applied biosystems
by life technologies

Real-Time PCR Systems
TaqMan® Protein Assays
Chemistry Guide

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Contents

About This Guide

Overview

This guide is designed to give researchers using TaqMan® Protein Assays reagents the following:

- Background information on proximity ligation technology and TaqMan Protein Assays reagents (Chapter 1)
- Supplemental information on relative quantitation and screening experiments with TaqMan Protein Assays reagents (Chapter 2)
- Background information on the proteins targeted by TaqMan Protein Assays, with example data (Chapter 3)

Detailed instructions for using the TaqMan Protein Assays reagents and related products described in this guide are provided in these protocols:

- *TaqMan® Protein Assays Probe Development Protocol* (PN 4449282)
- *TaqMan® Protein Assays Sample Prep and Assay Protocol* (PN 4449283)

Safety information

For general safety information, see this section and Appendix B, "Safety" on page 59.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 60.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

1 Introduction to TaqMan® Protein Assays

This chapter covers:

About proximity ligation technology with TaqMan® Protein Assays reagents

Introduction

The TaqMan® Protein Assays reagents enable detection and relative quantitation of proteins in cultured mammalian cell and tissue lysates. The reagents use an adapted form of PLA™, a proximity ligation assay technology, in combination with real-time PCR.

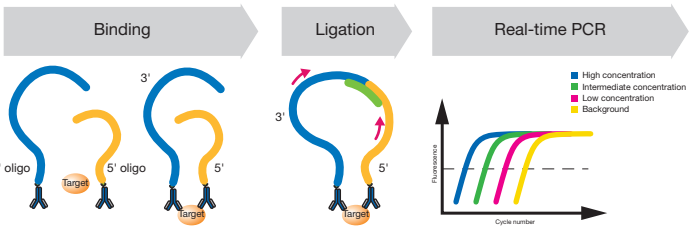
Proximity ligation is a technology for converting specific antibody binding events into reporter nucleic acid molecules. PLA combines antibody-protein binding with detection of the reporter nucleic acid by real-time PCR. As illustrated in Figure 1 on page 10, the basic steps are:

1. **Binding of a protein target by paired assay probes.**
   The assay probes are target-specific antibodies that are conjugated to oligonucleotides through a biotin-streptavidin linkage. Each oligonucleotide in the probe pair presents a 5′ or 3′ end; these ends are brought into proximity when the antibody components of the assay probe pair concurrently bind to two different epitopes on the target protein.

2. **Ligation of the oligonucleotides by DNA ligase.**
   The substrate for ligase is a bridge structure formed by hybridization of a third oligonucleotide to the oligonucleotide ends of the assay probe pair. This structure forms preferentially when the assay probes are in proximity to each other. Subsequent protease treatment inactivates the ligase.

3. **Amplification and detection of the ligation product by TaqMan® real-time PCR.**

Figure 1 TaqMan® Protein Assays: Proximity ligation with real-time PCR



Overview of the TaqMan® Protein Assays procedure

The TaqMan® Protein Assays reagents enable detection of proteins via proximity ligation in a homogeneous solution format, with user-supplied cultured mammalian cell and tissue lysates and Applied Biosystems real-time PCR systems. The TaqMan Protein Assays procedure includes the steps shown in the workflow below.

Note: This section briefly describes the steps listed below; for a complete protocol, refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*. For a complete list of TaqMan Protein Assays reagents, see Appendix A on page 53.

(Optional) Develop Assay Probes (page 12)

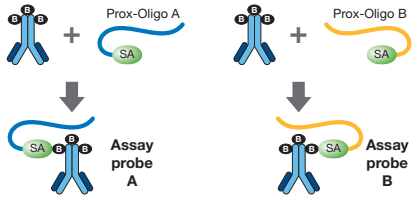
Select and prepare biotinylated antibodies

↓

Perform the Forced Proximity Probe Test

↓

Prepare the Assay Probes



↓

Prepare cell lysates (page 13)

↓

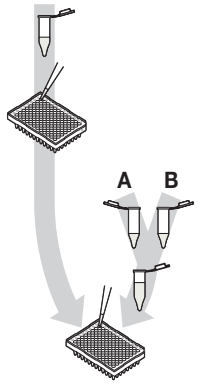
Perform the binding reaction (page 13)

Prepare the Assay Probe solution

↓

Prepare and incubate the binding reaction plate

↓



Perform the ligation and protease reactions (page 13)

Perform the ligation reaction

↓

Perform the protease reaction

↓

Perform the real-time PCR (page 13)

Prepare the PCR mix

↓

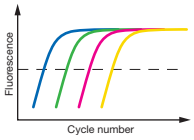
Prepare the PCR plate

↓

Run the PCR plate

↓

Analyze the real-time data (page 14)



(Optional) Develop Assay Probes

Used in conjunction, the kits listed below enable you to develop Assay Probes from your own biotinylated antibodies. After developing the Assay Probes, you can use them in TaqMan® Protein Assays experiments.

- **TaqMan® Protein Assays Oligo Probe Kit**
- **TaqMan® Protein Assays Buffer Kit**
- **TaqMan® Protein Assays Core Reagents Base Kit**
- **TaqMan® Protein Assays Fast Master Mix**

See Chapter 4 on page 41 for information on selecting your own biotinylated antibodies. For a complete protocol, refer to the *TaqMan® Protein Assays Probe Development Protocol*.

Prepare cell lysates

To prepare cell lysates that are optimally compatible for use with the TaqMan® Protein Assays, Applied Biosystems recommends one of the following kits:

- **Protein Expression Sample Preparation Kit**
- **Protein Quant Sample Lysis Kit**

The differences between the two kits are described in the table below.

Note: For information on using other sample lysis reagents, refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

	Protein Expression Sample Preparation Kit	Protein Quant Sample Lysis Kit
Sample type	Cultured mammalian cells	
Sample size	Useful for samples in limiting amounts: ~10,000 to 50,000 cells	>50,000 cells
Lysis reagent contains carrier protein	Yes	No
Method used to normalize data for TaqMan® Protein Assays analysis†	Cell count	Cell count or total protein concentration
Recommended cell lysate concentration	500 cells/µL	2500 cells/µL
Required initial dilution in the TaqMan® Protein Assay	1:2	1:10

† For the Protein Expression Sample Preparation Kit, it is important to count the cells accurately before lysis because the real-time PCR data is normalized to the number of cells used for each assay. For the Protein Quant Sample Lysis Kit, cell counting is optional but recommended.

Perform the binding reaction

A dilution series of the cell lysate is incubated for 60 minutes at 37 °C with assay probes (antibodies conjugated to 5′ and 3′ oligonucleotides) supplied with the **TaqMan® Protein Assays Kit** of choice. Each kit supplies paired assay probes targeting the protein of interest. See Chapter 3 for details about the proteins targeted by available assay probes.

Perform the ligation and protease reactions

After binding, ligation reaction components supplied with the **TaqMan® Protein Assays Core Reagents Base Kit** are added. The ligation reaction is incubated for 10 minutes at 37 °C, followed by a **Protease** treatment for 10 minutes at 37 °C. The protease is inactivated by heating at 95 °C for 5 minutes.

Perform the real-time PCR

Regardless of the protein target, every pair of TaqMan Protein Assays probes generates an identical ligation product which can then be amplified using the **Universal PCR Assay**. The ligation reaction products are transferred to a PCR plate and mixed with the Universal PCR Assay and **TaqMan® Protein Assays Fast Master Mix**. The plate is ready for real-time PCR on one of the following Applied Biosystems systems:

- ViiA™ 7 Real-Time PCR System
- StepOnePlus™ Real-Time PCR System
- 7900HT Fast Real-Time PCR System
- 7900HT Real-Time PCR System
- 7500 Fast Real-Time PCR System

Analyze the real-time data

Use the real-time PCR system software to obtain C_T values. For further analysis, you can export the real-time PCR data from 96- and 384-well reaction plates:

- For relative quantitation experiments, import the data into the ProteinAssist™ Software. The ProteinAssist Software is available at:

  www.appliedbiosystems.com/taqman4protein
- For screening experiments, import the data into a spreadsheet application, such as Microsoft® Excel® Software.

For further details about data analysis and experiment design using TaqMan Protein Assays reagents, refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

How TaqMan® Protein Assays differ from traditional real-time PCR

Traditional real-time PCR

In traditional real-time PCR, the instrument monitors the progress of the PCR as it occurs, via hydrolysis of the fluorogenic TaqMan® probe (5′ nuclease assay). Reactions are characterized by the PCR cycle at which the fluorescent amplification signal for the target crosses a threshold (threshold cycle; C_T). The higher the starting template concentration, the earlier in the reaction target amplification is detected, and the lower its C_T value. For more information, refer to the *Real-Time PCR Systems Chemistry Guide*.

TaqMan® Protein Assays

TaqMan Protein Assays data is also expressed as C_T values that are derived from the real-time PCR component of the assay. However, because the C_T values also reflect the assay probe binding and ligation events, relative quantitation of protein targets with TaqMan Protein Assays uses a different approach from relative quantitation of mRNA or DNA targets via traditional real-time PCR. Key features of this approach are described below.

C_T values are normalized to cell count or total protein concentration

With real-time PCR of mRNA targets, C_T values are routinely normalized to an endogenous control to correct for sampling variation and biological sample variability. However, there are currently no suitable endogenous controls available for TaqMan Protein Assays. The C_T values in TaqMan Protein Assays are instead normalized to cell count or total protein concentration, depending on the sample prep kit that you use:

- **Protein Expression Sample Preparation Kit** – Use this kit to normalize the C_T values to cell count.
- **Protein Quant Sample Lysis Kit** – Use this kit to normalize the C_T values to cell count or to total protein concentration.

For information on using both kits, refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

IMPORTANT! Applied Biosystems highly recommends that you obtain cell counts before lysis. If obtaining cell counts or protein concentration is not feasible, the cell equivalent concentration of the lysate can be estimated by quantitating its genomic DNA content, using TaqMan® RNase P Detection Reagents.

No Protein Controls are essential

Because some background ligation occurs in the absence of protein (Figure 2, 0 cell input; Fredriksson *et al.*, 2007), TaqMan Protein Assays must always include a No Protein Control (NPC) within the same plate. It is advantageous to calculate and plot the data as ΔC_T vs cell input, as shown in Figure 3, where:

$$\Delta C_T(X \text{ cell input}) = C_T(\text{NPC}) - C_T(X)$$

Data that is corrected for background can then be compared from different plates.

The linear dynamic range of the assay must be determined

When C_T values from TaqMan Protein Assays are plotted as a function of cell equivalents of cell lysate (expressed as cell input), a sigmoidal curve typically results (Figure 2). At high cell inputs, the plots typically exhibit a "hook" effect (Figures 2 and 3) that is frequently present in immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), and occurs at high ligand concentrations (Colby, 1999; Diamandis and Christopoulous, 1996). The linear dynamic range is the region within the curve that is suitable for relative quantification. The ProteinAssist Software automatically determines this range as the Regression Linear Range for each sample.

Figure 2 Cystatin B expression in Raji Protein Expression Lysate Control Kit: C_T vs cell input

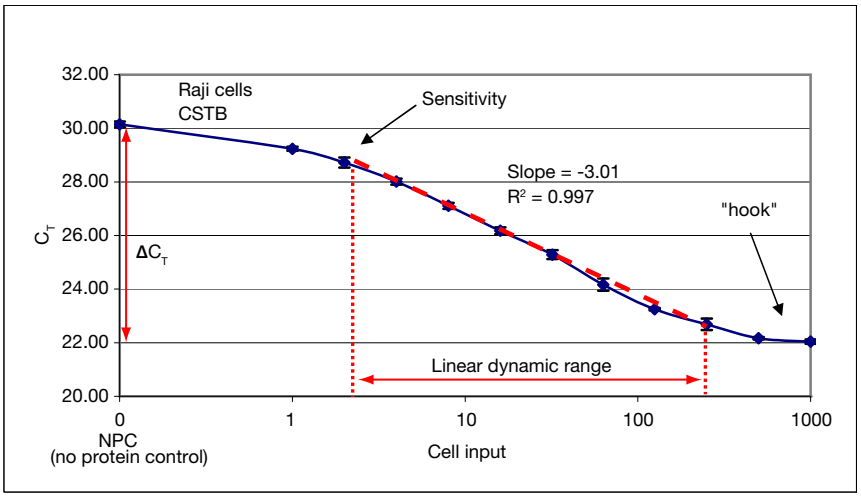
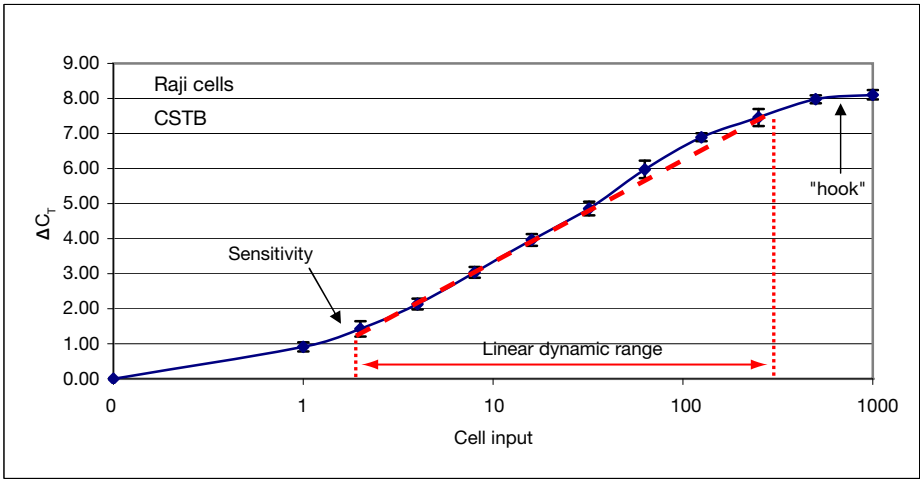


Figure 3 Cystatin B expression in Raji Protein Expression Lysate Control Kit: ΔC_T vs cell input



The assay efficiency has multiple kinetic components

Relative quantitation calculations in traditional real-time PCR are valid only when all samples have the same amplification efficiencies (Livak and Schmittgen, 2001). The amplification efficiency of a real-time PCR assay is measured by performing real-time PCR on a dilution series of the starting nucleic acid template and plotting C_T vs log(nucleic acid concentration). Such a plot forms a straight line, and the efficiency is then calculated from its slope:

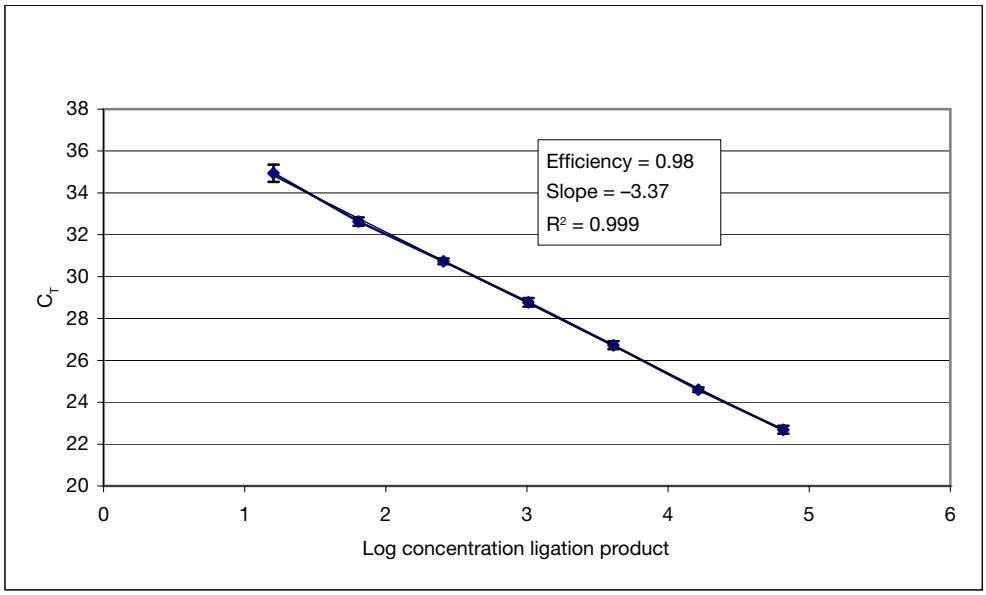
$$E = 10^{(-1/\text{slope})} - 1$$

The slope of such a plot for a PCR reaction with 100% efficiency (that is, the concentration of amplicon doubles with each cycle) is –3.3.

The sigmoidal curves generated from TaqMan Protein Assays (Figures 2 and 3) are in contrast to the straight-line plots typically derived from a nucleic acid dilution series. In addition, the slope of the linear range of the TaqMan Protein Assays cystatin B assay shown in Figure 2 is –3.01, which would correspond to an efficiency of 115% using the above efficiency calculation. This result indicates that the slope of the linear range of TaqMan Protein Assays is not solely dependent on PCR efficiency: it has a contribution from the protein-antibody binding dynamic as well.

The efficiency of the real-time PCR ***component*** of TaqMan Protein Assays can be measured with a dilution series of the ligation products of a ***single*** binding/ligation reaction. In the experiment shown in Figure 4, the ligation products generated during a single binding/ligation reaction were diluted and amplified using the Universal PCR Assay. This dilution series shows the same straight-line relationship seen with real-time PCR of a nucleic acid template, and it has a slope of –3.37, demonstrating that the real-time PCR component of TaqMan Protein Assays measures is ~100% efficient.

Figure 4 Efficiency of the real-time PCR component of TaqMan® Protein Assays



Because the slope of the linear range of TaqMan Protein Assays depends on multiple kinetic components, the slope of a dilution series may vary from sample to sample. Thus TaqMan Protein Assays cannot use standard real-time PCR data analysis methods, which depend on all samples having the same assay efficiency. These kinetic differences are taken into account by the ProteinAssist Software.

Background information on proximity ligation assay technology

General background

Immunodetection methods such as ELISA, Western blots, and immuno-PCR (Niemeyer *et al.*, 2007) rely on a single antibody-binding event. In contrast, proximity ligation technology requires that antibodies concurrently bind to two different epitopes of the same protein. The requirement for two independent binding events reduces background signal derived from nonspecific or cross-reactive antibody binding and contributes significantly to the high specificity and sensitivity of PLA technology (Gustafsdottir *et al.*, 2006; Söderberg *et al.*, 2007; Landegren *et al.*, 2004). Assay performance also depends on the affinity and specificity of the antibody for its target protein, because subsaturating amounts of antibody are typically used to minimize background (Gullberg *et al.*, 2004).

Real-time PCR amplification allows detection of virtually any ligation products that are formed during the assay. Consequently, proximity ligation technology with real-time PCR detection is several orders of magnitude more sensitive than Western technology (Gustafsdottir *et al.*, 2005; Gustafsdottir *et al.*, 2006).

Early forms of proximity ligation technology used DNA aptamers as the protein-binding affinity agent (Fredriksson *et al.*, 2002). Affinity probes have also been developed using peptide-phycoerythrin-oligonucleotide conjugates (Pai *et al.*, 2005). Methods for conjugation of oligonucleotides to polyclonal antibodies or to matched pairs of monoclonal antibodies have enabled development of antibody-based assay probes, such as those included in the TaqMan® Protein Assays Kits (Gullberg *et al.*, 2004; Fredriksson *et al.*, 2008; Gufstafsdottir *et al.*, 2006).

Proximity ligation assay formats

Proximity ligation assays have been demonstrated in the following formats:

- **Homogeneous** – Binding, ligation, and amplification occur in solution (Fredriksson *et al.*, 2002; Gullberg *et al.*, 2004). A homogeneous format, such as that incorporated into TaqMan Protein Assays, is amenable to small sample volumes and streamlined workflows.
- **Solid-phase** – The target protein is first immobilized on a solid-phase support using a capture antibody, then detected with a proximity ligation assay for the captured protein (Fredriksson *et al.*, 2002; Gustafsdottir *et al.*, 2006).
- **In situ** – The connector oligonucleotide generates a circular DNA strand upon hybridization to the paired proximity probes, followed by ligation. Rolling circle amplification by phi29 DNA polymerase of the circular template, which remains hybridized to the proximity probes, results in localized amplification of the ligation product (Söderberg *et al.*, 2006). This technology has been commercialized as Duolink® by Olink Bioscience (**www.olink.com**).
- **Multiplex homogeneous** – Proximity probes are constructed so that each target-specific ligation product is uniquely tagged for real-time PCR (Fredriksson *et al.*, 2007).

Applications of proximity ligation technology

The utility of proximity ligation assay technology has been demonstrated in a variety of experimental systems, using both homogeneous and in situ formats. A selection of recent studies is listed in Table 1.

Table 1 Selected applications of proximity ligation technology

Application	Reference
Detection and quantification of proteins	
Cytokine detection in mammalian cell culture and tissues	Gullberg *et al.*, 2004
Expanding applications of protein analysis using proximity ligation and qPCR	Swartzman *et al.*, 2010
Plasma biomarkers for pancreatic and ovarian cancer	Fredriksson *et al.*, 2008
Platelet-derived growth factor-B in glomerular preparations	Bjarnegård *et al.*, 2004
Active prostate-specific antigen	Zhu *et al.*, 2006
Intact porcine parvovirus (PPV) and *Lawsonia intracellularis*	Gustafsdottir *et al.*, 2006
Intact *Bacillus* spores	Pai *et al.*, 2005
Protein-protein interactions	
Prox1 – COUP-TFII	Yamazaki *et al.*, 2009
CA125 – mesothelin	Fredriksson *et al.*, 2008
c-Myc – Max	Soderberg *et al.*, 2006
VEGF-R2 – PDGF-Rβ	Greenberg *et al.*, 2008
Protein kinase B – inositol 1,4,5-trisphosphate receptor	Szado *et al.*, 2008
DNA-protein interactions	
Transcription factors p53, HNF-4, and USF1	Gustafsdottir *et al.*, 2007

2 Using TaqMan® Protein Assays

TaqMan® Protein Assays can be used for relative quantitation of protein targets with respect to reference samples, and for screening large numbers of samples in a semiquantitative assay. This chapter covers:

General experiment design

As described in Chapter 1, both protein-antibody binding events and real-time PCR amplification contribute to the kinetics of TaqMan Protein Assays. Therefore relative quantitation of protein targets with TaqMan Protein Assays uses a different approach from relative quantitation of mRNA or DNA targets using traditional real-time PCR. It is important to plan your experiments so that appropriate controls and reference samples are included for downstream data analysis.

The recommendations in this section apply to both relative quantitation and screening experiments.

Choose an appropriate reference sample

It is important for data analysis to choose a reference sample that is appropriate to your experimental needs.

- Relative quantitation and screening experiments with TaqMan Protein Assays both look at the change in abundance of a protein of interest in test samples relative to a reference sample.
- In relative quantitation experiments, the reference sample is used as a calibrator for calculating the fold change in expression of the protein of interest. For reliable data analysis, the reference sample should have a robust level of expression.

Examples of reference samples include:

- Untreated cells, for a drug or chemical treatment study
- Time zero cells, for a time course study
- A cell line for which it is known (by independent measurements) that the abundance level of the protein of interest is unchanged under the experimental conditions

Normalize data to cell count or total protein concentration

Because there are currently no suitable endogenous controls for TaqMan Protein Assays, data is normalized to cell count or total protein concentration.

It is important to obtain accurate cell counts or total protein concentration for both relative quantitation and screening experiments:

- In relative quantitation experiments with TaqMan Protein Assays, C_T values are normalized to cell count or total protein concentration.
- In screening experiments with TaqMan Protein Assays, multiple samples are compared to the reference sample at the same cell or total protein concentration.

For information on determining cell count with cytometric methods or by estimating genomic DNA content, refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

Include No Protein Controls in each assay plate

Because some background ligation occurs in the absence of cell extract, include No Protein Control (NPC) assays to measure the assay background.

- In place of cell lysate, use the buffer that is in included in one of the kits listed below:

Kit	Buffer†
TaqMan® Protein Assays Buffer Kit	Lysate Dilution Buffer
Protein Expression Lysate Control Kit	
Protein Expression Sample Preparation Kit	Cell Resuspension Buffer

† The buffer formulations in each kit are identical; the buffers can be used interchangeably.

- Include NPCs on ***each*** assay plate.
- You can use the same NPC wells for both reference and test samples.

In relative quantitation with TaqMan Protein Assays, the C_T value of each sample is corrected for the NPC background; this corrected value is called ΔC_T. (This definition of ΔC_T differs from that used in traditional real-time PCR, where ΔC_T is the C_T value of a sample normalized with respect to the endogenous control.)

In screening experiments, it is not necessary to calculate a ΔC_T value. The NPC can be used as a negative control, or strictly as a measure of assay background values.

Relative quantitation experiments

Experiment design

Test a dilution series of each cell extract

When TaqMan Protein Assays C_T values for an individual cell lysate sample are plotted as a function of log[cell input (that is, cell equivalents per assay)], a sigmoidal curve typically results (for example, see Figure 2 on page 15). The linear region of the curve defines the range of the assay for that lysate that is suitable for relative quantitation. However, the linear range and its slope may be different for each individual lysate, due to the contribution of the antibody-protein binding step to the assay kinetics. These kinetic differences must be taken into account during data analysis, therefore the linear range must be determined for each lysate.

Plan to assay a dilution series of the cell lysate prepared from ***each*** sample in your experiment, including the reference sample. The ProteinAssist™ Software determines the best-fit regression line for each dilution series. Follow these guidelines:

- For robust data analysis, a minimum of four dilution points for each cell lysate is recommended, although the ProteinAssist Software allows as few as two dilutions of each cell lysate.
- Include NPC wells on each plate. You can use the same NPC wells for both reference and test lysates.
- It can be useful to first perform TaqMan Protein Assays on a 2- to 3-fold dilution series of the reference cell lysate in your experimental system, to see the full dynamic range of the assay for the protein target in your experimental system. For a cell lysate with an initial concentration of ~500 cells/µL, the dilution series would require six to ten points. Based on this initial data, you may choose to assay fewer dilutions of both reference and test lysates. For instance, 3-fold dilutions, or 2-fold dilutions over a narrower cell input range, may still yield robust data.

Include three replicates for each dilution point

To obtain robust data, plan for three replicates for each point in the dilution series, as described in the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

Example experiment

The relative quantitation example experiment tests the effect of trans-retinoic acid (tRA) treatment on LIN28 protein levels in NTERA2 cells. This example experiment focuses on:

- Experiment design (this page)
- Data analysis of the experiment using the ProteinAssist Software (page 23)

The details of the assay procedure are covered in the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

Experiment design

NTERA2 cells were treated on Day 0 with tRA. On Day 0 and on days 14 and 28 after treatment, cells were harvested, counted using a hemocytometer, and lysed using the Protein Expression Sample Preparation Kit, as described in the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

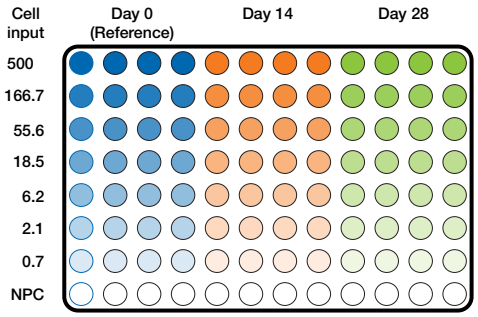
Each cell extract was first diluted 2-fold to 250 cell equivalents per µL. A 3-fold dilution series was then prepared from this working stock, and 2 µL of each dilution was assayed in quadruplicate with the TaqMan Protein Assays LIN28 assay, the TaqMan® Protein Assays Core Reagents Base Kit, and the TaqMan® Protein Assays Fast Master Mix, as described in the *TaqMan® Protein Assays Sample Prep and Assay Protocol* (500 cell equivalents to 0.7 cell equivalents per assay).

For this experiment, the goal was to determine the amount of LIN28 on days 14 and 28 relative to day 0, therefore the experiment was designed as follows:

- Reference sample – day 0 (untreated) cells
- Test samples – days 14 and 28 cells
- Total of three cell extracts

Figure 5 on page 23 shows a typical assay plate layout for this type of experiment.

Figure 5 Example assay plate layout for tRA experiment



Using the ProteinAssist™ Software

The ProteinAssist Software is designed for analysis of relative quantitation experiments with TaqMan Protein Assays. The software is available for download at:

www.appliedbiosystems.com/taqman4protein

Minimum requirements for the experiment file

To be successfully imported into the ProteinAssist Software, the experiment files that you create in your real-time PCR system software must:

- Be compatible with the ProteinAssist Software. You can import experiment files that were created with the following real-time PCR systems:

Applied Biosystems Real-Time PCR System	Software version	Experiment file extension
7500 Fast system	7500 Software v2.0.2, v2.0.3, and v2.0.4	*.eds
	SDS Software v1.4	*.csv
7900HT Fast system	SDS Software v2.3 (Patch A, B, C)	*.txt
StepOnePlus™ system	StepOne™ Software v2.1	*.csv, *.eds, *.txt
ViiA™ 7 system	ViiA™ 7 Software v1.0	*.csv, *.txt

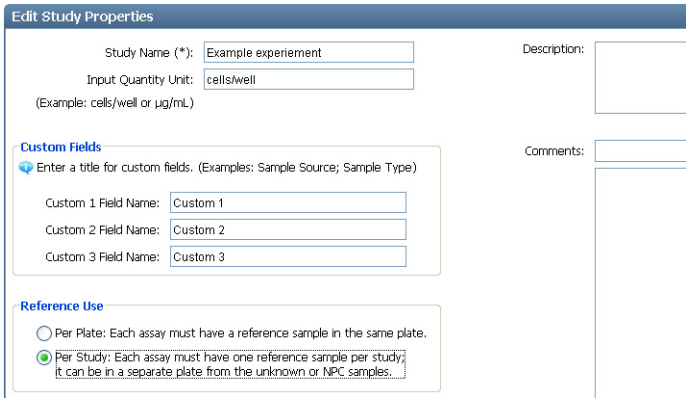
Note: For detailed procedures on creating and exporting experiment files, refer to the documentation for your real-time PCR system.

- Contain C_T values. For some instruments, the experiment file may also include sample name, input quantity, or assay name.

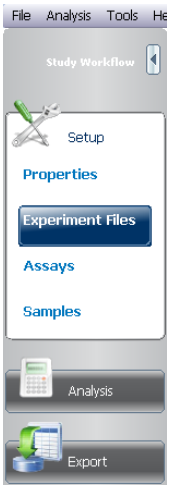
Workflow for the example experiment

The table below shows the ProteinAssist Software workflow steps for the example experiment.

Note: For detailed procedures and information about the software features, refer to the *ProteinAssist™ Software Getting Started Guide*. The Getting Started Guide is included in the ProteinAssist Software download.

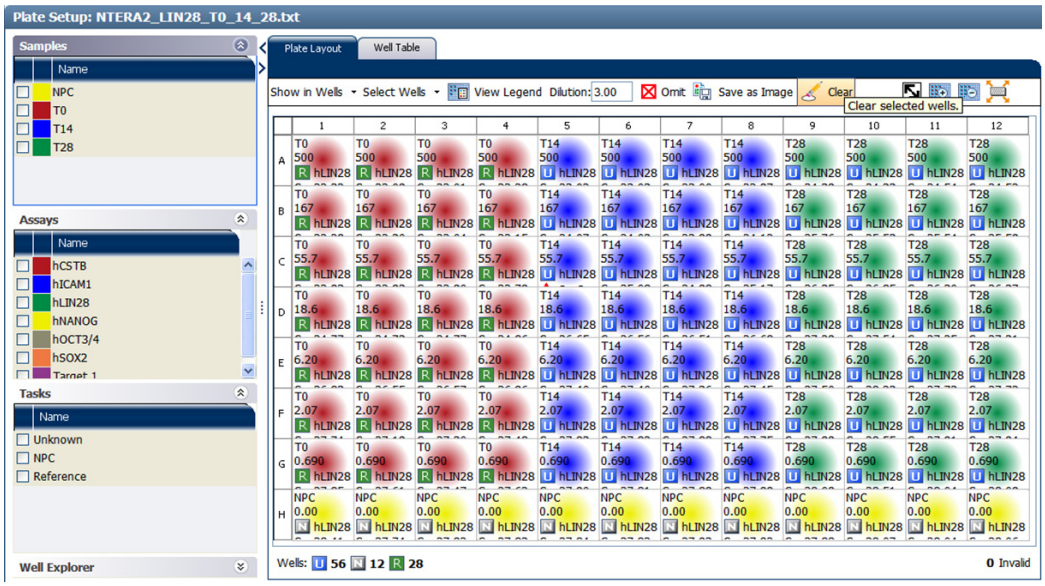
Workflow step	Key tasks completed for the example experiment
Step 1: Set up a study.	
	• Input Quantity Unit – **cells/well** • Reference Use – **Per Study** 

Workflow step	Key tasks completed for the example experiment
Step 2: Set up the experiment file within the study.	



- Imported experiment file – One *.txt file from the StepOnePlus™ Real-Time PCR system.
- Required well attributes:
  - Input Quantity – **500**
  - Sample names and tasks – **T0/Reference**, **T14/Unknown**, **T28/Unknown**, and **NPC/NPC**
  - Assay name – **hLIN28**
- Dilution factor – **3.00** (3-fold)

IMPORTANT! When you create your own study, note that each experiment file in a study must follow specific analysis rules. To view the analysis rules, click the **Plate Setup Help** icon (Plate Setup Help) in the Experiments toolbar.

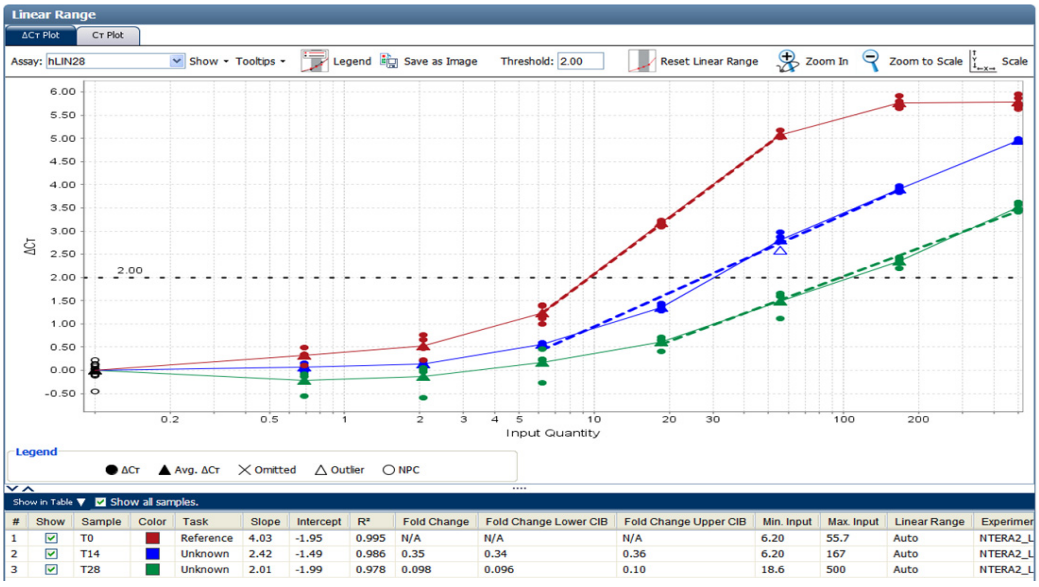


Workflow step	Key tasks completed for the example experiment
Step 3: Analyzed the study and viewed the analysis results.	

In the Linear Range screen, ΔC_T values are displayed in graph and table formats, and fold change results are displayed in table format.

The ProteinAssist Software automatically determines a set of data for each sample that follows a linear relationship between log(input quantity) and ΔC_T. This set of data is used in the relative quantification calculation. For each sample, a dotted line shows the regression line for that set of data.

The figure below displays the results for the example experiment. The regression line for the reference sample (T0) is shown in red. The table format and the graph show that the linear ranges and slopes of the regression lines for the reference sample (T0-red), day 14 sample (T14-blue), and day 28 sample (T28-green) are all different.



#	Show	Sample	Color	Task	Slope	Intercept	R²	Fold Change	Fold Change Lower CIB	Fold Change Upper CIB	Min. Input	Max. Input	Linear Range	Experimer
1	☑	T0		Reference	4.03	-1.95	0.995	N/A	N/A	N/A	6.20	55.7	Auto	NTERA2_L
2	☑	T14		Unknown	2.42	-1.49	0.986	0.35	0.34	0.36	6.20	167	Auto	NTERA2_L
3	☑	T28		Unknown	2.01	-1.99	0.978	0.098	0.096	0.10	18.6	500	Auto	NTERA2_L

(Optional) In the Linear Range screen, you can modify the data as follows:

- Manually set the linear range.
- Adjust the threshold.
- Change the analysis settings.
- Examine the data for outliers, then omit wells.

Workflow step	Key tasks completed for the example experiment
	In the Fold Change screen, fold change results are displayed in bar graph and table formats. The ProteinAssist Software calculates a fold change for the test samples relative to the reference sample/calibrator. Fold change values <1 indicate decreased protein expression relative to the reference sample. The figure below displays the results for the example experiment.  (Optional) In the Fold Change screen, you can modify the data as follows: • Change the graph format (Log_2, Log_{10}, or linear). • Change the analysis settings.

Screening experiments

Experiment design

Compare samples at the same cell or total protein input

To use TaqMan Protein Assays for screening purposes, compare test samples to a reference sample at one or two lysate concentrations. This strategy is useful for indicating whether there is increased or decreased expression of the protein of interest, although it does not result in data that can be used for relative quantitation calculations.

Experimental conditions which result in increased expression of the target protein may give C_T values in the hook region of the TaqMan Protein Assays (this hook effect is seen at high ligand concentrations in many immunoassays; Figure 2 on page 15). In a screening experiment performed at one lysate concentration per sample, such increased-expression hits could be missed. To avoid this possibility, it is best to use two lysate concentrations for each sample.

To identify the lysate concentration(s) for screening experiments:

1. Perform TaqMan Protein Assays for the protein target of interest on a 2- to 3-fold dilution series of an appropriate reference lysate, starting at:
   - 500 cell equivalents per well
   - 50 ng of total protein per well, for cell lysates
   - 1000 ng of total protein per well, for tissue lysates

   Prepare a reference sample for each cell line in your screening experiment.

2. Choose one or two lysate concentrations within the linear range of the dilution series of the reference lysate for your screening experiment. Use the same lysate concentrations for the test and reference samples.

When screening large numbers of samples, you might choose to use the TaqMan® RNase P Detection Reagents to estimate cell counts and adjust the concentration of each cell lysate so that the test and reference samples are assayed at the same cell equivalent input amount(s). (Note that the RNase P method cannot be used to determine absolute cell count.) Refer to the *TaqMan® Protein Assays Sample Prep and Assay Protocol*.

Include a minimum of two replicates of each assay

Prepare a minimum of two (ideally three) replicate TaqMan Protein Assays for each sample dilution.

Example experiment

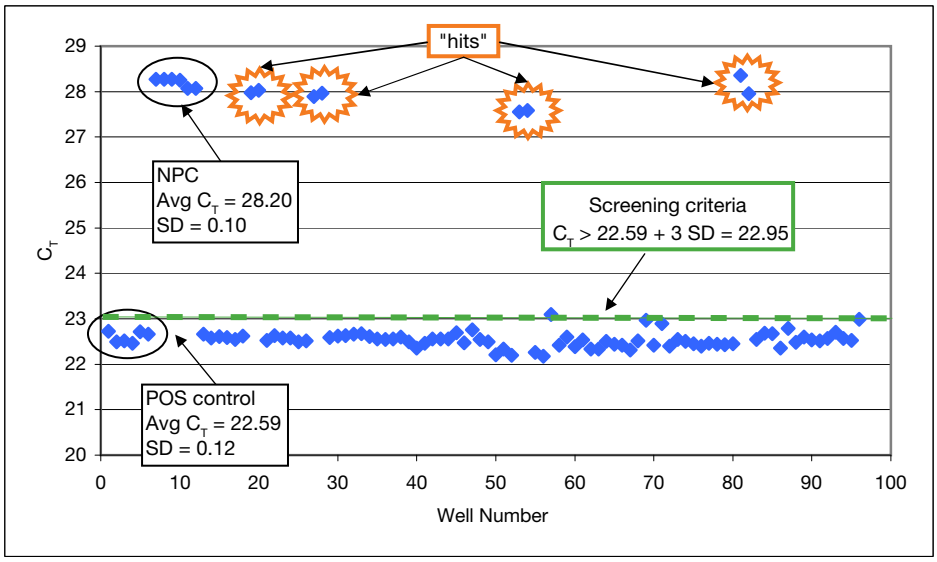
The experiment shown in Figure 6 is a demonstration of what a screening experiment with TaqMan Protein Assays might look like.

LIN28 is highly expressed in pluripotent cell lines, and its expression is repressed upon commitment to cellular differentiation. The TaqMan Protein Assays LIN28 assay can be used to screen for small molecules, such as trans-retinoic acid (tRA), that induce differentiation in a pluripotent cell line such as NTERA2, resulting in decreased LIN28 expression. Most of the samples in Figure 6 are from untreated cells, but four samples ("hits") are from cells treated with tRA for 28 days.

In this example experiment, the middle of the Regression Linear Range for the reference lysate (untreated cells, day 0) was determined to be at 50 cells per assay (data not shown). Therefore all screening assays were performed at this input.

- Reference sample – Untreated cell lysate, 6 replicate assays; this sample also serves as a positive control for LIN28 expression
- Test samples – Treated cell lysates, 2 replicate assays per lysate
- No Protein Control – Cell Resuspension Buffer, 6 replicate assays; this sample also serves as a negative control and background measurement

Figure 6 LIN28 expression in NTERA2 cells: sample screening experiment



Data analysis

Data analysis for screening experiments is handled with spreadsheet software. Export the data from your real-time PCR instrument to a tab-delimited (*.txt) or comma-separated values (*.csv) format file that is compatible with Excel software or another spreadsheet program.

You can set the criteria for identifying a hit using the desired confidence interval around the average C_T of the appropriate reference sample:

- 99% confidence interval = Avg C_T ± 3 SD
- 95% confidence interval = Avg C_T ± 2 SD

In the screening experiment shown in Figure 6, the criteria used for identifying a hit was a C_T value outside the 99% confidence interval for the average C_T of the positive control (untreated cells). The example hits fall well outside this interval. These samples can be further evaluated through a relative quantitation experiment with the TaqMan Protein Assays LIN28 assay.

TaqMan® Protein Assays: best practices

Table 2 Best practices: Dos

Practice	Reason or details
Measure the ***same*** protein target in test and reference samples.	TaqMan® Protein Assays are validated only for this type of comparison.
Include a No Protein Control (NPC) for each assay on each plate.	Background ligation events measured in the NPC are subtracted by the ProteinAssist™ Software to generate ΔC_T values.
Plan for the appropriate number of replicate TaqMan Protein Assays.	• Relative quantitation experiments – a minimum of three replicates of each dilution of the cell lysate. • Screening experiments – a minimum of two, ideally three, replicates of each lysate.
Relative quantitation experiments: If you are normalizing to cell count (rather than total protein concentration): • Obtain an accurate cell count. • Plan for normalization to cell input.	The ProteinAssist™ Software is designed to normalize ΔC_T values to cell input. (There is currently no suitable endogenous control for TaqMan Protein Assays.)
Relative quantitation experiments: Plan for the appropriate number of dilutions of each lysate.	A minimum of four dilutions of each lysate is recommended, plus the NPC. • The same NPC wells are used for both reference and test lysates. • To identify the linear range for your protein target, perform TaqMan Protein Assays on a 2- to 4-fold dilution series of the reference lysate, starting with: – 500 cells/µL if you used the Protein Expression Sample Preparation Kit – 2500 cells/µL or 250 ng/µL if you used the Protein Quant Sample Lysis Kit – 5000 ng/µL for tissue lysates Based on this data, plan the dilutions for your experiment.
Screening experiments: • Compare all samples at the same lysate concentrations. • Use two lysate concentrations per sample.	Use two lysate concentrations, to avoid missing hits with increased expression due to the hook effect of the assay. To determine the appropriate cell or tissue input for screening: 1. Perform TaqMan Protein Assays for the protein target of interest on a 2- to 3-fold dilution series of an appropriate reference lysate, starting with: – 500 cells/µL if you used the Protein Expression Sample Preparation Kit – 2500 cells/µL or 250 ng/µL if you used the Protein Quant Sample Lysis Kit – 5000 ng/µL for tissue lysates 2. Choose one or two lysate concentrations within the linear range of the reference lysate for your screening experiment.

Practice	Reason or details
Run the PCR plate immediately after completing the reaction setup.	• After the PCR mix has been mixed with the protease-treated ligation product, you should run the PCR plate immediately. If necessary, you can store the PCR plate on ice or at room temperature for up to 4 hours. • The protease-treated ligation product itself can be stored at 4 °C for up to 3 days, or at –20 °C for up to 2 weeks.
Use ICAM1 and CSTB TaqMan Protein Assays for control experiments and troubleshooting, not as an endogenous control.	These protein markers have not been validated for use as endogenous controls.
For preparation of cell lysates *and* real-time PCR with TaqMan® RNase P Detection Reagents, follow good PCR practices.	Because PCR can detect only a few copies of target, it is crucial to avoid contamination of samples that will undergo PCR amplification. • Maintain separate areas and dedicated equipment and supplies for: – Sample preparation – PCR setup – PCR amplification – Analysis of PCR products • Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (PN AM9890). • Use a positive-displacement pipette or aerosol-resistant pipette tips. • Follow proper dispensing techniques to prevent aerosols. • Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation). • Change gloves whenever you suspect that they are contaminated. • Never bring amplified PCR products into the PCR setup area. • Centrifuge sample tubes before opening. Open and close sample tubes carefully. Try not to splash or spray PCR samples. • Keep reactions and components capped as much as possible.

Table 3 Best practices: Don'ts

Practice	Reason or details
Do not use Protein Expression Lysate Control Kits as the reference lysate.	• The reference lysate should be a sample that is appropriate as a calibrator in your experimental system. • The control lysates are not validated as calibrators. • Use the Protein Expression Lysate Control Kits only for control experiments and troubleshooting.
Do not use the ICAM1 and CSTB markers as endogenous controls.	• These markers have not been validated as endogenous controls • The ProteinAssist™ Software does not normalize to endogenous controls, only to cell or total protein input. • Use these markers when performing control experiments with Protein Expression Lysate Control Kits.

3 Background on Protein Targets and Cell Lines

Overview

TaqMan® Protein Assays kits provide ready-made assay probes for detecting selected protein targets by proximity ligation technology. TaqMan Protein Assays kits are available for:

- Human pluripotency markers LIN28, NANOG, OCT3/4, and SOX2. Background information starts on page 34.
- Human control markers CSTB and ICAM1, for control experiments and troubleshooting. Background information starts on page 37.

Protein Expression Lysate Control Kits provide cell lysates prepared from cell lines known to express proteins targeted by available assay probes:

- NTERA2 (human embryonal carcinoma cell line, pluripotent)
- Raji (human lymphoma cell line, non-stem cell)

These control lysates are designed for use in control TaqMan Protein Assays and for troubleshooting. Background information about the cell lines used for the Protein Expression Lysate Control Kits starts on page 37.

Together, the TaqMan Protein Assays Kit and the Protein Expression Lysate Control Kits provide a system for both positive and negative controls for detection of pluripotency markers in your experimental system, as summarized in the table below.

Table 4 Detection of human markers in Protein Expression Lysate Control Kits

TaqMan® Protein Assays		Protein Expression Lysate Control Kits	
		NTERA2	Raji
Human pluripotency markers	LIN28	+	–
	NANOG	+	–
	OCT3/4	+	–
	SOX2	+	–
Human control markers	CSTB	+	+
	ICAM1	+	+

Example data for available TaqMan Protein Assays protein markers under a variety of experimental conditions starts on page 38.

Human pluripotency markers

General background

Pluripotency is the ability of a cell to develop into any of the cell lineages of the body (endoderm, mesoderm, or ectoderm). Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass (ICM) of the developing blastocyst. ES cells can be propagated indefinitely in culture, but under suitable culture conditions they will differentiate into major cell lineages.

Certain types of human and mouse somatic cells can be reprogrammed to a pluripotent state upon introduction of genes known to be critical for maintenance of pluripotency (Yu *et al.*, 2007; Nakagawa *et al.*, 2008; Jaenisch and Young, 2008; Takahashi and Yamanaka, 2006). These induced pluripotent stem (iPS) cells behave similarly to ES cells in a variety of characteristics, including the ability to differentiate into various cell lineages.

Understanding maintenance of pluripotency and commitment to cell differentiation on a molecular level is crucial to understanding developmental processes and to exploiting the therapeutic potential of pluripotent cells. The pluripotency markers described in this section play critical roles in establishment or maintenance of pluripotency in mammalian cells. The TaqMan Protein Assays kits can be used for accurate confirmation of the presence of these pluripotency markers or for characterization of "stemness" in cell lines of interest.

NANOG, OCT4, and SOX2: background

NANOG, OCT4, and SOX2 are each essential for maintenance of pluripotency in ES cells, and they can be used as markers for pluripotency. NANOG, OCT4, and SOX2 are transcription factors that may work together as master regulators of cell development in mammalian cells (Jaenisch and Young, 2008).

- NANOG is a homeodomain transcription factor, characterized by a conserved DNA-binding region of ~60 amino acids (Pan and Thomson, 2007).
- OCT4 (Octamer-4; OCT3/4; POU5F1) is a member of the POU (Pit-Oct-Unc) family of homeodomain transcription factors (Pan *et al.*, 2002). POU proteins contain a homeodomain and a structurally analogous DNA-binding POU domain, and they are thought to regulate transcription through binding an octameric sequence motif at their target genes.

  The *OCT4* gene clearly has a role in maintenance of pluripotency (Nichols *et al.*, 1998; Niwa *et al.*, 2000). However, analysis has been complicated by the finding that the human *OCT4* gene potentially encodes several protein isoforms. Initially, two splice variants were identified that encoded protein isoforms OCT4A and OCT4B (Takeda *et al.*, 1992). OCT4A and OCT4B share identical POU and C-terminal domains but differ in their N termini. OCT4A is localized in the nucleus and is associated with maintenance of pluripotency. The role of OCT4B, which does not stimulate transcription of OCT4-dependent promoters and is localized to the cytoplasm, is not clear (Lee *et al.*, 2006). Analysis of expression studies of *OCT4* is further complicated by the presence of multiple pseudogenes (Liedtke *et al.*, 2007; Liedtke *et al.*, 2008). Recently an additional splice variant was identified, OCT4B1, that is highly expressed in human ES/EC cells, suggesting a potential

role in maintenance of pluripotency (Atlasi *et al.*, 2008; Papamichos *et al.*, 2009). Multiple translation start sites of the OCT4B mRNA have been shown to encode three protein isoforms. One of these isoforms, OCT4B-190, may play a role in the cellular stress response (Wang *et al.*, 2009).

- SOX2 (SRY [sex determining region Y]-box 2) is a member of the B1 group of the *SOX* gene family of developmental transcription factors in human and mouse; this gene family is characterized by a highly conserved high mobility group (HMG) domain (Schepers *et al.*, 2002). SOX2 can heterodimerize with OCT3/4; interaction of HMG domains (such as in SOX2) and homeodomains (such as in OCT4) is thought to be an important mechanism for regulation of gene expression during development (Boyer *et al.*, 2005; Dailey and Basilico, 2001).

Viral-mediated transduction of *SOX2*, *OCT4*, *NANOG*, and *LIN28* constructs has been shown to be sufficient to cause reprogramming of human fetal and postnatal fibroblasts to iPS cells; *NANOG* and *LIN28* increased the efficiency of reprogramming but were not absolutely required (Yu *et al.*, 2007). Transduction of *SOX2*, *OCT3/4*, and *KLF4* (Kruppel-like factor 4 transcription regulator) has also been shown to reprogram adult human fibroblasts into iPS cells (Nakagawa *et al.*, 2008). Transient expression of the transduced genes appears to initiate the reprogramming, but stable iPS cells are characterized by subsequent activation of endogenous pluripotency genes. Activation of the endogenous genes is thought to be the result of stochastic epigenetic events (Jaenisch and Young, 2008).

Global characterization of gene targets for NANOG, OCT4, and SOX2 (by chromatin immunoprecipitation combined with microarray analysis or paired-end ditag sequencing) in human and mouse cells has shown that these three proteins co-occupy or bind to a large number of genes (Boyer *et al.*, 2005; Loh *et al.*, 2006).

One class of genes co-occupied by NANOG, OCT4, and SOX2 is active in ES cells, and it includes genes encoding transcription factors, signal transduction components, and chromatin-modifying proteins. NANOG, OCT4, and SOX2 also bind to their own promoters and to the promoters of the other two genes, forming an autoregulatory loop. Thus these three proteins may promote pluripotency through positive regulation of key pathways for pluripotency as well as their own genes (Jaenisch and Young, 2008; Boyer *et al.*, 2005; Masui *et al.*, 2007).

Another large class of genes co-occupied by NANOG, OCT4, and SOX2 is silenced or repressed in ES cells, and this class includes genes that are developmental transcription factors. The mechanism of silencing involves Polycomb Group (PcG) proteins; PcG proteins are conserved from *Drosophila* to humans and are thought to maintain gene silencing through histone methylation and condensation of chromatin structure. An additional form of transcriptional regulation of NANOG/OCT4/SOX2 gene targets involves RNA Polymerase II that has initiated transcription but is unable to elongate, perhaps due to activity of the PcG apparatus. Thus, these gene targets, although silenced in stem cells, appear to be poised for transcription activation during differentiation (Jaenisch and Young, 2008).

Using the NANOG, OCT3/4, and SOX2 assays

NANOG, OCT4, and SOX2 are highly expressed in ICM and undifferentiated ES cells. Use the NTERA2 Protein Expression Lysate Control Kit when performing a positive control assay with TaqMan Protein Assays NANOG, OCT3/4, and SOX2 assays (Figure 7A, B, and C). The Raji Protein Expression Lysate Control Kit can be used as a negative control lysate for these markers, for troubleshooting purposes. Note that the antibody assay probe in the OCT3/4 assay recognizes both OCT4A and OCT4B isoforms.

LIN28: background

LIN28 can be used as a marker for pluripotency in human cells. It is one of four trans-acting factors that collectively are sufficient to reprogram human somatic fibroblasts to iPS cells (Yu *et al.*, 2007; see also "NANOG, OCT4, and SOX2: background" on page 34).

Human and mouse LIN28 plays a key role in maturation of Let-7 miRNA, a highly conserved miRNA that is critical to regulation of cell proliferation and differentiation. LIN28 has been shown in vitro and in vivo to selectively block Drosha-mediated processing of the primary transcripts (pri-miRNAs) of Let-7 family miRNAs in mammalian cells (Bussing *et al.*, 2008; Viswanathan *et al.*, 2008; Newman *et al.*, 2008).

LIN28 contains a cold-shock domain and two zinc finger domains, a unique domain organization shared by all known RNA binding proteins. Binding of LIN28 to the stem-loop region of the Let-7 pri-miRNA transcript is integral to inhibition of Let-7 pri-miRNA processing (Newman *et al.*, 2008). A LIN28 isoform lacking the cold-shock domain is ineffective at inhibition of pri-miRNA processing (Viswanathan *et al.*, 2008).

In reprogramming experiments with *SOX2*, *OCT4*, *NANOG*, and *LIN28* constructs, transduction of *LIN28* improved the efficiency of iPS clone recovery but was not absolutely required (Yu *et al.*, 2007). A different combination of factors, *OCT4*, *SOX2*, *KLF4* and *c-MYC*, has also been shown to be sufficient to reprogram human somatic cells to iPS (Lowry *et al.*, 2008; Park *et al.*, 2008; Takahashi *et al.*, 2007). Because MYC is a negative transcription regulator of *Let-7* family genes, a link between the roles of MYC and LIN28 in reprogramming has been speculated. Whether the link is through regulation of Let-7 miRNA maturation by LIN28 (Bussing *et al.*, 2008) or by some other mechanism, such as recruitment or activation of endogenous MYC proteins (Nakagawa *et al.*, 2008), remains to be determined.

Using the LIN28 assay

Human LIN28 is highly expressed in undifferentiated ES cells. Use the NTERA2 Protein Expression Lysate Control Kit when performing a positive control assay with the TaqMan Protein Assays human LIN28 assay (Figure 7D). The Raji Protein Expression Lysate Control Kit can be used as a negative control lysate for this marker, for troubleshooting purposes.

Human control markers

CSTB: background

CSTB (Cystatin B) is a cytoplasmic cysteine protease inhibitor that is found in many cell types. CSTB is thought to protect cells by regulating the activity of its target proteases (Turk *et al.*, 2008).

ICAM1: background

ICAM1 (Intercellular adhesion molecule 1; also known as CD54) is a transmembrane protein that is found on the cell surface of many cell types. ICAM1 functions in cell-cell interactions and leukocyte adhesion (Wittchen, 2009). It has binding sites for numerous ligands, most notably a number of immune-associated ligands. Its function in intercellular adhesion appears to be part of a broader role in signal transduction associated primarily with inflammatory response pathways (Lawson and Wolf, 2009).

Using the CSTB and ICAM1 assays

CSTB and ICAM1 are expressed in many cell types. Use either the Raji or the NTERA2 Protein Expression Control Lysate Kit when performing a positive control assay with TaqMan Protein Assays CSTB and ICAM1 assays (Figure 7E and F).

Protein Expression Lysate Control Kits

NTERA2 Kit: background

The NTERA-2 cl.D1cell line used for the NTERA2 Protein Expression Lysate Control Kit is a pluripotent human embryonal carcinoma cell line (ATCC® Number CRL-1973™). These cells can be induced to differentiate along neuroectodermal lineages upon exposure to retinoic acid or hexamethylene bisacetamide (Andrews, 1988; Andrews, 1984). The NTERA2 Protein Expression Lysate Control Kit provides a stem-cell line lysate that can be used in positive control assays with TaqMan Protein Assays kits for all the pluripotency and control markers listed in Table 4 on page 33.

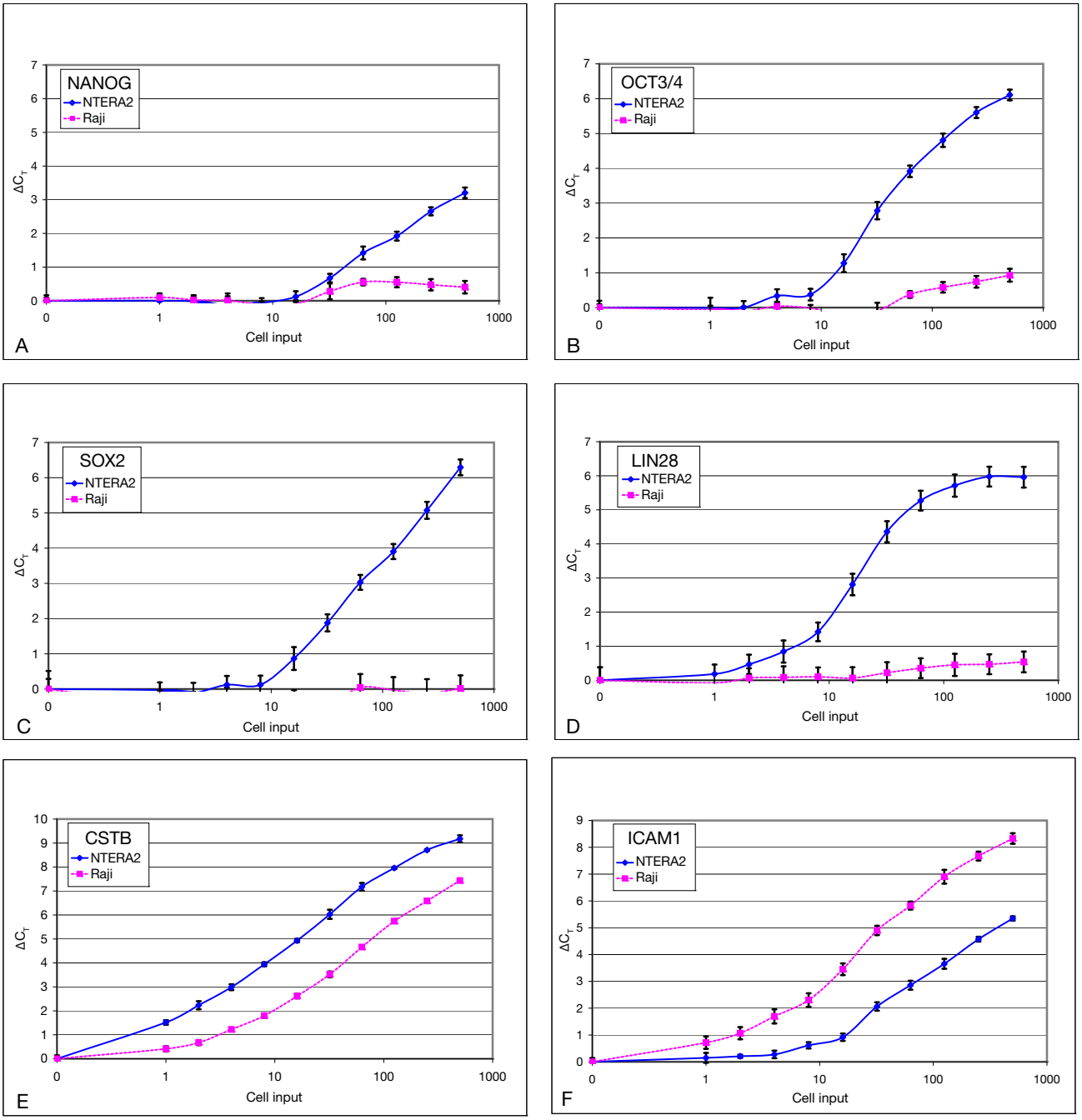
Raji Kit: background

The Raji cell line used for the Raji Protein Expression Lysate Control Kit is a lymphoblast-like cell line derived from a Burkitt's lymphoma (ATCC® Number CCL-86™). The Raji Protein Expression Lysate Control Kit provides a non-stem-cell line lysate that can be used in positive control assays with TaqMan Protein Assays CSTB and ICAM1 assays (Table 4)

Example data

Figure 7 shows typical data for each pluripotency or control marker using the NTERA2 or Raji Protein Expression Lysate Control Kit. Each point is the average of four replicate TaqMan Protein Assays.

Figure 7 Example data: TaqMan® Protein Assays with Protein Expression Lysate Control Kits



Other experimental conditions

TaqMan Protein Assays for human pluripotency and control markers have been used in a variety of cell lines and experimental conditions. These results are summarized in Tables 5 and 6.

Table 5 Detection of human pluripotency markers using TaqMan® Protein Assays (In the table, a + indicates positive detection of the protein marker.)

Type of cell line	Name	Description	LIN28	NANOG	OCT3/4	SOX2
Human stem cell lines	NTERA2	embryonal carcinoma	+	+	+	+
	2102Ep	embryonal carcinoma	+	+	+	+
	NCCIT	pluripotent stem cell	+	+	+	+
	BG01V	embryonic stem cell line	+	+	+	+
	SA002	embryonic stem cell line	+	+	+	+
Human non-stem cell lines	Raji	lymphoma	–	–	–	–
	TCam-2	seminoma cell line	+	+	+	–
	MCF7	breast cancer	–	–	–	–
	FS-1	testicular somatic	–	–	–	–
	PC-3	prostate cancer	–	–	–	–
Mouse pluripotent cell line	P19	mouse embryonal carcinoma	–	–	–	–
Hamster non-stem cell line	CHO	Chinese hamster ovary cell line	–	–	–	–

Table 6 Use of TaqMan® Protein Assays in a variety of experimental conditions

Treatment	Description	Cell line	Protein markers tested	Protein expression results
tRA	Induces differentiation	NTERA2	LIN28, NANOG, OCT3/4, SOX2	All markers: decreased
siRNA	gene knockdown of OCT3/4	NTERA2	LIN28, NANOG, OCT3/4, SOX2	OCT3/4: decreased LIN28, NANOG, SOX2: unchanged
Recombinant protein expression	NANOG, OCT3/4, SOX2	CHO	LIN28, NANOG	NANOG: • Abundant in cells overexpressing NANOG. • Undetectable in cells overexpressing OCT3/4, or SOX2. LIN28: Undetectable in cells overexpressing NANOG, OCT3/4, or SOX2.

4 Designing Assays with User-Supplied Antibodies

This chapter covers:

Target selection

Successful TaqMan Protein Assay results have been obtained with protein targets ranging in molecular size from 6 kDa to >200 kDa. When you prepare samples with the Protein Quant Sample Lysis Kit or the Protein Expression Sample Preparation Kit, a whole cell lysate is generated, which enables detection of membrane-bound, cytosolic, and nuclear-localized proteins. Successful assays have been created for a variety of functional protein classes; for example:

- Transcription factors
- Cytokines
- Cell surface proteins, including membrane-bound receptors
- Receptor ligands
- Signal transduction pathway proteins
- Growth factors
- Protein tags, such as GFP (green fluorescent protein) and GST (glutathione-S-transferase)

Additional applications include:

- Phosphorylated-site detection on pathway response proteins
- Detection of protein-protein interactions in both homomeric and heteromeric complexes
- Detection of cell surface markers on suspensions of intact mammalian cells

Antibody selection

TaqMan® Protein Assays can be developed for a wide variety of targets with user-supplied biotinylated antibodies and the **TaqMan® Protein Assays Open Kit**. The Open Kit contains:

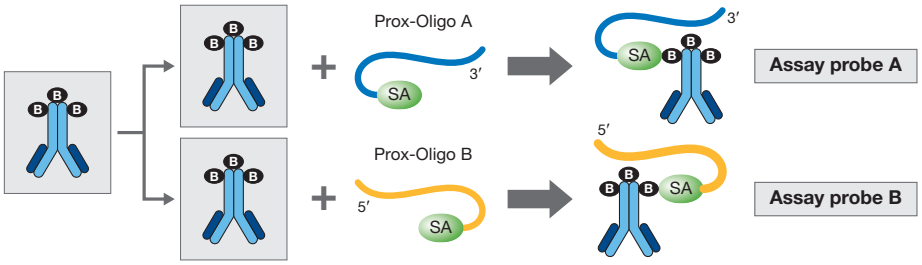
- 5′ and 3′ Prox-Oligos (streptavidin-linked oligos)
- Buffers for preparing the two Assay Probes used in the binding step of TaqMan Protein Assays experiments

TaqMan Protein Assays experiments require antibodies that bind to two or more distinct epitopes on the target protein to enable ligation of the 3′ and 5′ oligos.

Assay design options

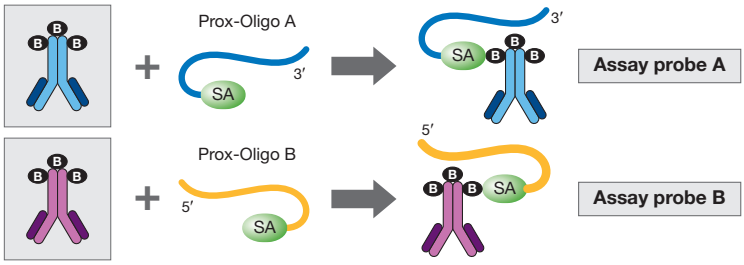
Depending on the availability of antibodies to your chosen target, there may be several assay design options:

- The simplest assay design uses a single polyclonal antibody that is split into two pools to generate the 3′ and 5′ oligo-linked antibodies (Assay Probes A and B):



It is important that the polyclonal antibody is raised against a full-length or near-full-length antigen (>100 aa). An Assay Probe pair made with a polyclonal antibody raised against a small peptide (<100 aa) will probably not be successful in the TaqMan Protein Assays experiment because it is likely that the antibody will bind only to a single epitope. A single monoclonal antibody is also not suitable for generating a pair of Assay Probes because it binds to a single epitope and cannot form the requisite proximity binding pair.

- An alternative assay design uses two different antibodies that have been qualified to function as a matched pair in an ELISA. The matched pair can be polyclonal/monoclonal, monoclonal/monoclonal, polyclonal/polyclonal.



The table below summarizes the assay design options.

Assay Probe pair configuration	Description	
Single antibody or matched antibody pair	Antigen-purified polyclonal	Raised against full-length or near-full-length antigen (>100 aa)
Matched antibody pair		Raised against peptide (<100 aa)
Matched antibody pair	Monoclonal	

IMPORTANT! The polyclonal antibodies used to make the Assay Probes must be antigen-purified. Antibodies that are not antigen-purified usually contain significant amounts of non-specific antibodies; non-specific antibodies will diminish the sensitivity and specificity of the assay.

Antibody search

Due to the inherent nature of antibodies, Applied Biosystems cannot guarantee that the antibody or antibody pair that you select will result in a working proximity probe set. Refer to the *TaqMan® Protein Assays Probe Development Protocol* for a list of commercially available antibodies that have been demonstrated to be either successful or unsuccessful in TaqMan Protein Assays experiments when tested with a suitable positive control. The overall success rate with these antibodies is 74%. To view the most current list, go to:

www.appliedbiosystems.com/taqman4protein

If you cannot find your target in the list of positively tested assays, and you do not yet have antibodies to try in TaqMan Protein Assays experiments, you may be able to find commercially available antibodies to your target by searching online at:

www.labome.com and **www.linscottsdirectory.com**

Biotinylated antibodies

The Assay Probes are prepared with biotinylated antibodies. If the antibody you select is not biotinylated, you must label the antibody with biotin and remove unreacted biotin using dialysis. The TaqMan Protein Assay is very sensitive to free biotin. As little as 80 nM of free biotin in the antibody preparation will diminish assay performance. Consequently, biotinylated antibody preparations must not contain any free biotin. Applied Biosystems strongly recommends that you perform the Forced Proximity Probe Test for all newly purchased or made biotinylated antibodies (see "About the Forced Proximity Probe Test" on page 45). The requirements for pre-biotinylated and non-biotinylated antibodies are summarized in the table below.

Antibody	Requirements	Recommended kits for biotinylation and dialysis	Forced Proximity Probe Test
Pre-biotinylated	Does not contain any free biotin	NA	Must pass the Forced Proximity Probe Test (ΔC_T >8.5)
Non-biotinylated	Free of carrier protein and in an amine-free buffer (for example, no BSA, gelatin, glycine, Tris, or azide)	• EZ-Link® Sulfo-NHS-LC-Biotin, No-Weigh™ Format (Pierce, PN 21327) • Biotin-XX Microscale Protein Labeling Kit (Molecular Probes, PN B30010)	
	Requires thorough dialysis to remove free biotin after the biotinylation reaction	Slide-A-Lyzer® Mini Dialysis Unit (MWCO=7000) (Thermo Scientific, PN 69562)	

About the Forced Proximity Probe Test

The Forced Proximity Probe Test determines whether or not the biotinylated antibody is suitable for making proximity probes. The test will fail if:

- Free biotin is in the preparation
- The antibody is under-biotinylated
- The biotinylated antibody is less concentrated than expected

Applied Biosystems recommends that you perform the Forced Proximity Probe Test for *all* biotinylated antibodies, whether the antibodies are user-biotinylated or pre-biotinylated by a vendor.

IMPORTANT! The Forced Proximity Probe Test determines whether or not the biotinylated antibody can bind to the Prox-Oligos. The test cannot determine whether or not the antibody is suitable for use in TaqMan Protein Assays experiments.

Assay Optimization

The TaqMan Protein Assay can be optimized at several stages of the protocol. You can:

- Decrease the Prox-Oligo volumes (this page)
- Reduce the Assay Probe concentrations in the binding reaction (page 47)
- Modify the binding reaction time and temperature (page 47)
- Include additives in the binding reaction buffer (page 47)
- Modify the sample preparation procedure (page 48)

Decrease the Prox-Oligo volumes

The following variation on preparing Assay Probes A and B uses less Prox-Oligos and may improve the signal:noise ratio of the assay. This variation is suggested for use only with polyclonal antibodies.

Follow the procedures in the *TaqMan® Protein Assays Probe Development Protocol* to prepare Assay Probes A and B, but use the volumes listed in the table below. The remainder of the Assay Probe preparation protocol is unchanged; the final volume of Assay Probes is 100 µL.

Prepare Assay Probe A

Component	Protocol volume (µL)	
	Standard	Modified
200 nM Biotinylated antibody	5.0	5.0
Antibody Dilution Buffer	NA	2.5
200 nM 3′ Prox-Oligo	5.0	2.5
Total volume	10.0	10.0

Prepare Assay Probe B

Component	Protocol volume (µL)	
	Standard	Modified
200 nM Biotinylated antibody	5.0	5.0
Antibody Dilution Buffer	NA	2.5
200 nM 5′ Prox-Oligo	5.0	2.5
Total volume	10.0	10.0

Reduce the Assay Probe concentrations in the binding reaction

You can reduce the concentration of the Assay Probes in the binding reaction to try to improve the signal:noise ratio. The concentration of each Assay Probe in the standard binding reaction is 250 pM. The table below provides suggested Assay Probe A and B volumes for preparing solutions with reduced Assay Probe concentrations.

Note: Applied Biosystems does not recommend using less than 50 pM of each Assay Probe in the binding reaction because the background signal (noise) of the assay will be too variable.

Order to combine	Component	Standard volumes	Modified volumes			
1	Assay Probe Dilution Buffer, 1X	216.0 µL	220.8 µL	225.6 µL	230.4 µL	235.2 µL
2	Assay Probe A (10 nM)	12.0 µL	9.6 µL	7.2 µL	4.8 µL	2.4 µL
3	Assay Probe B (10 nM)	12.0 µL	9.6 µL	7.2 µL	4.8 µL	2.4 µL
Total volume of Assay Probe solution		240.0 µL	240.0 µL	240.0 µL	240.0 µL	240.0 µL
Concentration of each Assay Probe in the Assay Probe solution		500 pM	400 pM	300 pM	200 pM	100 pM
Concentration of each Assay Probe in the binding reaction		250 pM	200 pM	150 pM	100 pM	50 pM

Modify the binding reaction time and temperature

The standard binding reaction is performed at 37 °C for 60 minutes. However, the signal:noise ratio for your assay may be improved by modifying the time and/or temperature of the binding reaction. Applied Biosystems suggests the following alternate binding reaction times and temperatures:

- 25 °C for several hours
- 4 °C for several hours to overnight

Include additives in the binding reaction buffer

The standard buffer used in the binding reaction is either the Lysate Dilution Buffer or the Cell Resuspension Buffer, depending on TaqMan Protein Assays kit that you use. The buffer formulations in each kit are identical; the buffers can be used interchangeably. The standard buffer contains PBS, 0.1% carrier protein, and other blocking agents.

To improve the signal:noise ratio of your assay, you may decide to include additives such as extra carrier protein (for example, gelatin or nonfat powdered milk). The ligation and qPCR reactions are not inhibited with binding buffers containing up to 0.5% gelatin or 5% nonfat powdered milk. BSA is not recommended.

Modify the sample preparation procedure

You may improve the signal:noise ratio by modifying your sample prep procedure and/or by using another lysis reagent. Appendix A in the *TaqMan® Protein Assays Sample Prep and Assay Protocol* lists alternate lysis reagents for cells and tissues that are known to be compatible with the TaqMan Protein Assays. Additional treatments, such as lysate clarification by centrifugation or heat treatment (for example, 75 °C for 10 minutes) may also help improve assay performance.

For more information

Refer to the following sections in the *TaqMan® Protein Assays Probe Development Protocol*:

For...	See...
A list of commercially available antibodies that have been demonstrated to be either successful or unsuccessful in TaqMan® Protein Assays when tested with a suitable positive control	Appendix A, "Screened Antibodies."
Guidelines on labeling non-biotinylated antibodies with biotin and removing free biotin by extensive dialysis	Appendix B, "Select and Prepare Non-Biotinylated Antibodies."
The Forced Proximity Probe Test procedures	"Perform the Forced Proximity Probe Test."
An illustration of how the performance of the TaqMan Protein Assays is severely compromised when even a small amount of free biotin is present	Appendix C, "Example Forced Proximity Probe Test and TaqMan® Protein Assays Data."

5 Using Controls

Using controls can be very helpful when you are developing a new TaqMan® Protein Assay or preparing new Assay Probes with the TaqMan® Protein Assays Open Kit. This chapter discusses the different controls that you can use at each stage of a TaqMan Protein Assays experiment.

This chapter covers:

TaqMan® Protein Assays general workflow

If you are new to TaqMan Protein Assays experiments, Applied Biosystems recommends that you familiarize yourself with the workflow using a control lysate (Raji) and pre-made Assay Probes (hCSTB):

Item	Applied Biosystems part number
TaqMan® Protein Assay Kit (hCSTB)	4405465
Protein Expression Lysate Control Kit (Raji)	4405448
TaqMan® Protein Assays Core Reagents Kit with Master Mix	4405501

Positive Assay for use with lysates from human cells or tissues

CSTB is ubiquitously expressed in most tissue or cell types and can serve as a positive control with lysates that you generate from human cells or tissues. Note that CSTB has not been validated as a useful endogenous control for relative quantification purposes. Applied Biosystems recommends that you include the Raji lysate as the positive control lysate.

Item	Applied Biosystems part number
TaqMan® Protein Assay Kit (hCSTB)	4405465
Protein Expression Lysate Control Kit (Raji)	4405448
TaqMan® Protein Assays Core Reagents Kit with Master Mix	4405501
One of the following sample prep kits:	
• Protein Quant Sample Lysis Kit	4448536
• Protein Expression Sample Prep Kit	4405443

Positive Assay for use with lysates from mouse embryonal stem cells or equivalent

Applied Biosystems does not have pre-made Assay Probes to mouse targets. However, the hSOX2 Assay Probes will moderately cross-react with mSOX2. Therefore, it may be possible to detect moderate to high levels of mSOX2 in mouse cells or tissues expressing mSOX2. The hSOX2 Assay Probes have successfully detected mSOX2 in P19 [ATCC CRL-1825] and F9 [ATCC CRL-1720] lysates.

Item	Applied Biosystems part number
TaqMan® Protein Assay Kit (hSOX2)	4405495
TaqMan® Protein Assays Core Reagents Kit with Master Mix	4405501
One of the following sample prep kits:	
• Protein Quant Sample Lysis Kit	4448536
• Protein Expression Sample Prep Kit	4405443

Positive Assay for use with lysates from normal mouse cells or tissues

Applied Biosystems has made mICAM1 Assay Probes using the TaqMan Protein Assays Open Kit and a biotinylated polyclonal mICAM1 antibody and successfully detected mICAM1 in a mouse universal tissue lysate. Therefore, the mICAM1 assay may be a suitable positive control for use with your mouse cell or tissue lysates. This positive control requires that you prepare your own mICAM1 Assay Probes with the Open Kit and the commercially available biotinylated polyclonal mICAM1 antibody. The mouse universal tissue lysate also serves as a useful positive control.

Item	Supplier	Part number
Biotinylated Anti-mouse ICAM-1 Antibody	R&D Systems	BAF796
Universal Protein Lysate: Mouse Normal Tissues	BioChain	P4334566
TaqMan® Protein Assays Open Kit	Applied Biosystems	4453745
TaqMan® Protein Core Reagents Kit with Master Mix	Applied Biosystems	4405501
One of the following sample prep kits:		
• Protein Quant Sample Lysis Kit	Applied Biosystems	4448536
• Protein Expression Sample Prep Kit	Applied Biosystems	4405443

Preparing Assay Probes with the Open Kit and your own biotinylated antibodies

When making Assay Probes with your own biotinylated antibody or antibody pair, it may be useful to generate an Assay Probe with a control biotinylated antibody to serve as a process control. The pre-made hCSTB Assay Probes from Applied Biosystems are prepared with a biotinylated anti-human CSTB polyclonal antibody from R&D Systems. You can prepare hCSTB Assay Probes with the biotinylated antibody and compare the performance of your hCSTB Assay Probes with the pre-made hCSTB Assay Probes from Applied Biosystems using the Raji lysate control.

Item	Supplier	Part number
Biotinylated Anti-human CSTB Antibody	R&D Systems	BAF1408
TaqMan® Protein Assay Kit (hCSTB)	Applied Biosystems	4405465
Protein Expression Lysate Control Kit (Raji)	Applied Biosystems	4405448
TaqMan® Protein Assays Open Kit	Applied Biosystems	4453745
TaqMan® Protein Core Reagents Kit with Master Mix	Applied Biosystems	4405501

Preparing Assay Probes with the Open Kit and your own non-biotinylated antibodies

You will need to biotinylate your antibody or antibody pair before making the Assay Probes. It may be useful to biotinylate a control antibody to serve as a process control. You can biotinylate the hCSTB antibody from R&D Systems and prepare Assay Probes with your biotinylated antibody, as well as with the biotinylated version of the same antibody. You can then compare the performance of those Assay Probes with the pre-made hCSTB Assay Probes from Applied Biosystems using the Raji lysate control.

Item	Supplier	Part number
Anti-human CSTB Antibody	R&D Systems	AF1408
Biotinylated Anti-human CSTB Antibody	R&D Systems	BAF1408
Slide-A-Lyzer® Mini Dialysis Unit (MWCO=7000)	Thermo Scientific	69562
One of the following labeling kits:		
• Biotin-XX Microscale Protein Labeling Kit	Molecular Probes	B30010
• EZ-Link® Sulfo-NHS-LC-Biotin, No-Weigh™ Format	Pierce	2132
TaqMan® Protein Assays Open Kit	Applied Biosystems	4453745
TaqMan® Protein Assays Kit (hCSTB)	Applied Biosystems	4405465
Protein Expression Lysate Control Kit (Raji)	Applied Biosystems	4405448
TaqMan® Protein Core Reagents Kit with Master Mix	Applied Biosystems	4405501

Using a mismatched Assay Probe pair as a negative control

You should always include a negative control in your experiment. It is best to use a sample that is known not to express the target of interest as your negative control. However, if no such sample exists (or if you want to use an additional negative control), you can use a mismatched Assay Probe pair.

For example, if your target is hCSTB, you can use a mismatched hCSTB/hICAM1 Assay Probe pair (hCSTB Assay Probe A + hICAM1 Assay Probe B). The mismatched Assay Probe pair should only generate background signal because both antibodies will not bind to the same target to enable proximity ligation of the 5′ and 3′ oligos.

A TaqMan® Protein Assays Reagents

How to order

The TaqMan® Protein Assays reagents are available from Applied Biosystems at:

www.appliedbiosystems.com

Kits for sample prep and assays

Sample preparation kits

Kit	Kit part number	Contents	Storage conditions
Protein Expression Sample Preparation Kit	4405443	Cell Lysis Reagent, 2X (3 × 1 mL)	2 to 8 °C
		Cell Resuspension Buffer (25 mL)	
Protein Quant Sample Lysis Kit	4448536	Sample Lysis Buffer (25 mL)	2 to 8 °C

Control kits

The Protein Expression Lysate Control Kits are for general use. You can use them for positive control reactions and for troubleshooting.

Kit	Kit part number	No. of reactions†	Contents	Storage conditions	
				Long-term	Short-term
Raji Kit; cells express ICAM1 and CTSB	4405448	100	Lysate Control, 500 cells/µL (50 µL)	< –50 °C	After initial use, store the **Lysate Dilution Buffer** at 2 to 8 °C for up to 3 months.
			Lysate Dilution Buffer (1 mL)	–15 to –25 °C‡	
NTERA2 Kit; cells express CSTB, ICAM1, LIN28, NANOG, OCT3/4, and SOX2	4405454	100	Lysate Control, 500 cells/µL (50 µL)	< –50 °C	
			Lysate Dilution Buffer (1 mL)	–15 to –25 °C‡	

† The number of reactions includes the reactions performed using the 2-fold dilution scheme outlined in this protocol.

‡ If necessary, the Lysate Dilution Buffer can be stored with the Lysate Control.

TaqMan® Protein Assays kits

Kit	Kit part number	No. of reactions	Contents	Storage conditions
Human CSTB Kit	4405465	100	• Assay Probe A, 20× (20 µL) • Assay Probe B, 20× (20 µL) • Assay Probe Dilution Buffer, 1× (0.5 mL)	–15 to –25 °C
Human ICAM1 Kit	4405471			
Human LIN28 Kit	4405477			
Human NANOG Kit	4405483			
Human OCT3/4 Kit	4405489			
Human SOX2 Kit	4405495			

Core reagents base kits

Kit	Part number		No. of reactions	Contents	Storage conditions	
	Kit	Top-fill†			Long-term	Short-term
TaqMan® Protein Assays Core Reagents Base Kit (100 rxn)	4405460	4405501	100	DNA Ligase, 500× (10 µL)	–15 to –25 °C	After initial use, store the **1× PBS** at 2 to 8 °C for up to 3 months.
				Ligase Dilution Buffer, 1× (2 × 1.5 mL)		
				Ligation Reaction Buffer, 20× (0.7 mL)		
				1× PBS, pH 7.4 (1 mL) (Phosphate Buffered Saline)		
				Protease, 100× (10 µL)		
				Universal PCR Assay, 20× (120 µL)		
TaqMan® Protein Assays Core Reagents Base Kit (500 rxn)	4448592	4448591	500	DNA Ligase, 500× (50 µL)	–15 to –25 °C	After initial use, store the **1× PBS** at 2 to 8 °C for up to 3 months.
				Ligase Dilution Buffer, 1× (15 mL)		
				Ligation Reaction Buffer, 20× (4 mL)		
				1× PBS, pH 7.4 (5 mL) (Phosphate Buffered Saline)		
				Protease, 100× (50 µL)		
				Universal PCR Assay, 20× (600 µL)		

† Top-fill part numbers include both the core reagents base kits and master mix.

Master mix

Kit	Part number		No. of reactions	Contents	Storage conditions
	Kit	Top-fill†			
TaqMan® Protein Assays Fast Master Mix, 2X (100 rxn)	4400088	4405501	100	Fast Master Mix, 2X (1.2 mL)	4 °C
TaqMan® Protein Assays Fast Master Mix, 2X (500 rxn)	4448616	4448591	500	Fast Master Mix, 2X (6 mL)	4 °C

† Top-fill part numbers include both the core reagents base kits and master mix.

Kits for probe development

Probe kits

Kit	Part number		No. of reactions	Contents	Storage conditions
	Kit	Top-fill†			
TaqMan® Protein Assays Oligo Probe Kit	4448549	4453745	4000	3′ Prox-Oligo, 200 nM (50 µL)	4 °C
				5′ Prox-Oligo, 200 nM (50 µL)	

† Top-fill part numbers include both the probe and buffer kits.

Buffer kits

Kit	Part number		No. of reactions	Contents	Storage conditions	
	Kit	Top-fill†			Long-term	Short-term
TaqMan® Protein Assays Buffer Kit	4448571	4453745	4000	Antibody Dilution Buffer (1 mL)	–15 to –25 °C	After initial use, store the **Assay Probe Dilution Buffer** and **Lysate Dilution Buffer** at 2 to 8 °C for up to 3 months.
				Assay Probe Storage Buffer (1 mL)		
				Assay Probe Dilution Buffer (7 mL)		
				Lysate Dilution Buffer (25 mL)		

† Top-fill part numbers include both the probe and buffer kits.

Core reagents base kits

Kit	Part number		No. of reactions	Contents	Storage conditions	
	Kit	Top-fill†			Long-term	Short-term
TaqMan® Protein Assays Core Reagents Base Kit (100 rxn)	4405460	4405501	100	DNA Ligase, 500X (10 µL)	–15 to –25 °C	After initial use, store the **1X PBS** at 2 to 8 °C for up to 3 months.
				Ligase Dilution Buffer, 1X (2 × 1.5 mL)		
				Ligation Reaction Buffer, 20X (0.7 mL)		
				1X PBS, pH 7.4 (1 mL) (Phosphate Buffered Saline)		
				Protease, 100X (10 µL)		
				Universal PCR Assay, 20X (120 µL)		
TaqMan® Protein Assays Core Reagents Base Kit (500 rxn)	4448592	4448591	500	DNA Ligase, 500X (50 µL)	–15 to –25 °C	After initial use, store the **1X PBS** at 2 to 8 °C for up to 3 months.
				Ligase Dilution Buffer, 1X (15 mL)		
				Ligation Reaction Buffer, 20X (4 mL)		
				1X PBS, pH 7.4 (5 mL) (Phosphate Buffered Saline)		
				Protease, 100X (50 µL)		
				Universal PCR Assay, 20X (600 µL)		

† Top-fill part numbers include both the core reagents base kits and master mix.

Master mix

Kit	Part number		No. of reactions	Contents	Storage conditions
	Kit	Top-fill†			
TaqMan® Protein Assays Fast Master Mix, 2X (100 rxn)	4400088	4405501	100	Fast Master Mix, 2X (1.2 mL)	4 °C
TaqMan® Protein Assays Fast Master Mix, 2X (500 rxn)	4448616	4448591	500	Fast Master Mix, 2X (6 mL)	4 °C

† Top-fill part numbers include both the core reagents base kits and master mix.

B

Appendix B Safety

This appendix covers:

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 60.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (**www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Documentation and Support

Documentation

TaqMan® Protein Assays documentation

Portable document format (PDF) versions of the documents listed below are available at **www.appliedbiosystems.com**

Note: To open the PDF versions, use the Adobe Acrobat Reader software available from **www.adobe.com**

Document	Part number
TaqMan® Protein Assays Sample Prep and Assay Protocol	4449283
TaqMan® Protein Assays Sample Prep Quick Reference Card	4449771
TaqMan® Protein Assays Assay Quick Reference Card	4449281
TaqMan® Protein Assays Probe Development Protocol	4449282
TaqMan® Protein Assays Probe Development Quick Reference Card	4449772
Real-Time PCR Systems TaqMan® Protein Assays Chemistry Guide	4405780
ProteinAssist™ Software Getting Started Guide	Included in the software download

Instrument documentation

To obtain the documents listed in this section or additional documentation, see "Obtaining support" on page 65.

7900HT/7900HT Fast system (Fast 96-Well, Standard 96-Well, or 384-Well Block Module)

Document	Part number
Applied Biosystems 7900HT Fast Real-Time PCR System Quick Reference Card: Performing Fast Gene Quantification	4351892
Applied Biosystems 7900HT Fast Real-Time PCR System Relative Quantitation Using Comparative C_T Getting Started Guide	4364016
Applied Biosystems 7900HT Fast Real-Time PCR System Absolute Quantification Getting Started Guide	4364014
Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4369584
Applied Biosystems 7900HT Fast Real-Time PCR System User Bulletin: Performing Fast Gene Quantification	4352533

7500 Fast system

Document	Part number
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Relative Quantification Getting Started Guide	4347824
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems Absolute Quantification Getting Started Guide	4347825
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments	4387779
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Getting Started Guide for Comparative C_T/Relative Standard Curve Experiments	4387783

StepOnePlus™ system

Document	Part number
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Reagent Guide	4379704
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Relative Standard Curve and Comparative C_T Experiments Getting Started Guide	4376785
Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems Getting Started Guide for Standard Curve Experiments	4376784

Chemistry/reagent guides

Document	Part number
Real-Time PCR Systems Chemistry Guide: Applied Biosystems 7900HT Fast Real-Time PCR Systems and 7300/7500/7500 Fast Real-Time PCR Systems	4348358
Applied Biosystems Real-Time PCR Systems Reagent Guide	4387787

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
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