

# TaqMan PreAmp Master Mix Kit

- Amplifies cDNA targets equally without introducing bias
- Multiplex up to 100 gene expression targets with minimal hands-on time
- Stretches 1 ng of cDNA into 200 real-time PCR reactions for gene expression analysis using Applied Biosystems™ TaqMan™ Gene Expression Assays
- Ideal for laser-capture microdissections, needle biopsies, and paraffin-embedded tissues



## Introduction

Gene expression analysis is often limited by the amount of RNA that can be extracted from the tissues or cells being studied. As more researchers turn to archived tissue samples such as formalin-fixed, paraffin-embedded (FFPE) tissues, laser-capture microdissection (LCM), and needle biopsies, sample size becomes a limiting factor in experimental design. To overcome this, methods to preamplify the RNA or cDNA have been developed. These methods, however, are limited in their effectiveness because they inconsistently amplify the starting material, resulting in biased and inaccurate amplification of some transcripts. Furthermore, these methods are usually laborious and time-intensive; some methods require more than 10 hours of manual steps and cycling time.

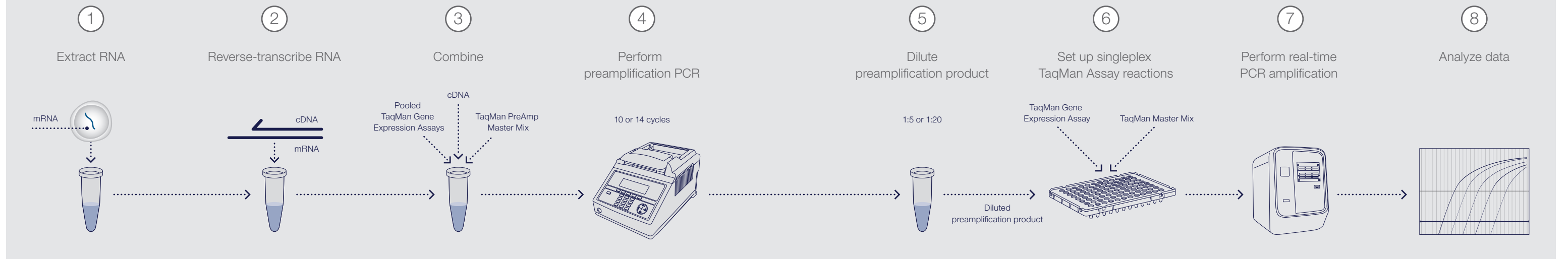
## TaqMan PreAmp Master Mix

The Applied Biosystems™ TaqMan™ PreAmp Master Mix preamplifies small amounts of cDNA without introducing amplification bias to the sample. Gene expression analysis of scarce cDNA is no longer inaccurate and labor-intensive. The TaqMan PreAmp Master Mix Kit uniformly enriches 1–250 ng of starting cDNA material for up to 100 gene-specific targets using a pool of TaqMan Gene Expression Assays as a source of primers. The PreAmp kit provides a simple, easy workflow and quantitative, reproducible results.

The standard real-time PCR reaction for gene expression analysis starts with the reverse transcription of total RNA to cDNA using random primers, followed by real-time PCR using gene-specific primers and probe. With TaqMan PreAmp Master

Mix, an intermediate multiplex step between reverse transcription and real-time PCR is performed in which the cDNA is enriched for up to 100 gene targets using a pool of TaqMan Gene Expression Assays (Figure 1). The preamplification reaction is cycled for 10 or 14 cycles to generate approximately 1,000- to 16,000-fold amplification of each gene-specific target (Table 1). The resulting preamplified reaction is diluted and serves as the starting material for the subsequent singleplex real-time PCR with each of the individual TaqMan Gene Expression Assays represented in the assay pool.

## Simple gene expression analysis workflow



**Figure 1. The complete workflow for analyzing gene expression using TaqMan PreAmp Master Mix.** The preamplification step takes only 15 additional minutes of hands-on time and 1.5 hours of cycling time.

### Uniform, unbiased amplification

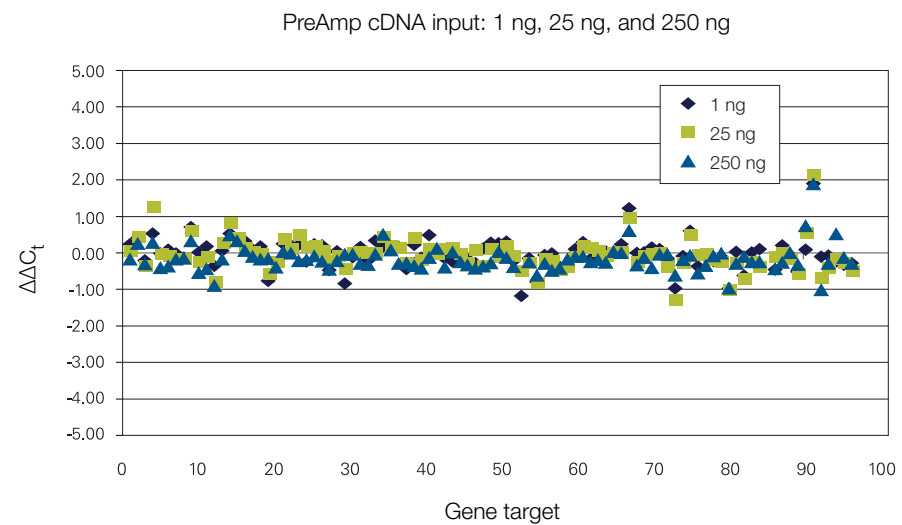
Current preamplification kits and techniques have been shown to generate poor correlation between preamplified and control (not preamplified) cDNA. Low correlation is caused by uneven target amplification, which results in nonquantitative, biased data. The  $\Delta\Delta C_t$  between a gene of interest and a normalizing gene, and between preamplified cDNA and control cDNA, can be as high as 2–5 cycles when using other commercially available preamplification kits.

TaqMan PreAmp Master Mix has been shown to produce virtually no difference in the  $\Delta\Delta C_t$  between preamplified cDNA from 1–250 ng of starting material and control (not preamplified) cDNA (Figure 2). For these preamplified targets, TaqMan PreAmp Master Mix provides extremely high correlation between the  $C_t$  values for cDNA and control cDNA for 1 ng and 25 ng of PreAmp starting material (Figure 3).

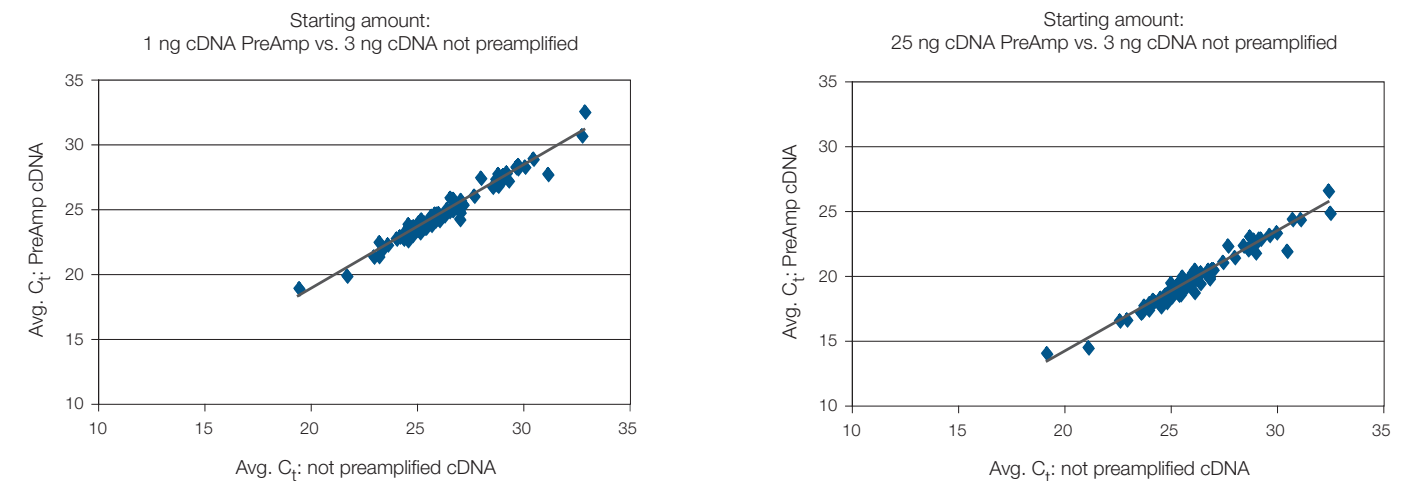
**Table 1. Amplification achieved using TaqMan PreAmp Master Mix.**

Starting material	Number of PreAmp PCR cycles	Dilution factor of preamplified cDNA	Number of real-time PCR reactions
1–250 ng cDNA	10 14	1:5 1:20	50 200

Choosing the number of cycles for adequate preamplification depends on the number of subsequent real-time PCR reactions that are desired.



**Figure 2. Survey of  $\Delta\Delta C_t$  values for various starting amounts of cDNA.** The  $\Delta\Delta C_t$  values for singleplex real-time PCR of preamplified cDNA (1, 25, and 250 ng of starting cDNA) vs. control (not preamplified) cDNA show minimal differences ( $\Delta\Delta C_t \leq 1$  for the majority of targets).



**Figure 3. Comparison of  $C_t$  values for amplification from different starting amounts of preamplified and not preamplified cDNA.** For both graphs,  $R^2 = 0.977$ .

Reliable and uniform preamplification enables researchers to analyze gene expression for limited quantities of cDNA samples from needle biopsies, laser-capture microdissections, and FFPE samples.

### A complete product suite for seamless workflow

The TaqMan PreAmp Master Mix Kit comes with TaqMan PreAmp Master Mix and TaqMan Gene Expression Master Mix. Both reagents work in tandem to provide optimal preamplification of cDNA. In addition

to the TaqMan PreAmp Master Mix Kit, other Applied Biosystems™ products required for successful preamplification of cDNA include:

- TaqMan Gene Expression Assays
- Applied Biosystems™ High Capacity cDNA Reverse Transcription Kit
- Applied Biosystems™ GeneAmp™ PCR System 9700
- Applied Biosystems™ 7500, ViiA™ 7, QuantStudio™, or StepOne™ Real-Time PCR Systems

Go to [thermofisher.com/mastermixes](http://thermofisher.com/mastermixes) for complete information on each of these integral products.

**Ordering information**

Product	Quantity	Cat. No.
<b>TaqMan PreAmp Master Mix Kit</b>		<b>4384267</b>
Components:		
1 mL tube of TaqMan PreAmp Master Mix	40 reactions (50 µL each)	4391128
5 mL bottle of 2X TaqMan Gene Expression Master Mix	200 reactions (50 µL each)	4369016
<b>Other required reagents</b>		
High Capacity cDNA Reverse Transcription Kit	200 reactions	4368814
TaqMan Gene Expression Assays	250 reactions (20 µL each) 360 reactions (20 µL each)	4331182 4351372
TaqMan Fast Advanced Master Mix*		
Mini-pack (1 x 1 mL)	100 reactions (20 µL each)	4444556
1-pack (1 x 5 mL)	500 reactions (20 µL each)	4444557
TaqMan Universal Master Mix II, with UNG*		
Mini-pack (1 x 1 mL)	40 reactions (50 µL each)	4440042
1-pack (1 x 5 mL)	200 reactions (50 µL each)	4440038
TaqMan Gene Expression Master Mix*		
Mini-pack (1 x 1 mL)	40 reactions (50 µL each)	4370048
1-pack (1 x 5 mL)	200 reactions (50 µL each)	4369016

\* User needs to choose only one of these three master mixes to use with the TaqMan PreAmp Master Mix Kit.

Find out more at [thermofisher.com/mastermixes](http://thermofisher.com/mastermixes)