

USER GUIDE

**ambion**<sup>®</sup>  
by *life* technologies™

# Cells-to-C<sub>T</sub><sup>™</sup> 1-Step *Power* SYBR<sup>®</sup> Green Kit

One-step RT-PCR in cell culture lysates

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**Catalog Number** A25601, A25600, A25599

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**Publication Number** MAN0010651

**Revision** A.0

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# About this guide

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**IMPORTANT!** Before using this product, read and understand the information in the “Safety” appendix in this document.

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## Revision history

Revision	Date	Description
A.0	June 2014	New document.



# Product information

## Product description

The Cells-to-C<sub>T</sub><sup>™</sup> 1-Step *Power* SYBR<sup>®</sup> Green Kit is designed for one-step reverse transcription and real-time PCR (real-time RT-PCR) in cell-culture lysates with SYBR<sup>®</sup> Green PCR technology. The kit includes reagents for cell lysis, optional DNase treatment, reverse transcription, and real-time PCR in pre-mixed formulations that minimize pipetting steps. PCR primers for the target of interest are provided by the user. Cells-to-C<sub>T</sub><sup>™</sup> 1-Step *Power* SYBR<sup>®</sup> Green Kit enables analysis of large numbers of samples with minimal risk of cross-contamination.

To provide a positive control for real-time RT-PCR and an indicator for the presence of RT-PCR inhibitors, RT-PCR targeting Xeno<sup>™</sup> RNA Control using template and primers from the SYBR<sup>®</sup> Green Cells-to-C<sub>T</sub><sup>™</sup> Control Kit can be performed. See “Perform 1-step RT-PCR with Xeno RNA Control” on page 15.

## Kit contents and storage

Table 1 Cells-to-C<sub>T</sub><sup>™</sup> 1-Step *Power* SYBR<sup>®</sup> Green Kit

Component	<a href="#">Buy Now</a>	<a href="#">Buy Now</a>	<a href="#">Buy Now</a>	Storage
	Cat. no. A25601 (20 rxns <sup>[1]</sup> )	Cat. no. A25600 (100 rxns <sup>[1]</sup> )	Cat. no. A25599 (400 rxns <sup>[1]</sup> )	
Lysis Solution	2.2 mL	5.5 mL	22 mL	2°C to 8°C <sup>[2]</sup>
DNase I	22 µL	55 µL	220 µL	-25°C to -15°C <sup>[3]</sup>
Stop Solution	200 µL	500 µL	2 × 1.1 mL	-25°C to -15°C <sup>[3]</sup>
Power SYBR <sup>®</sup> Green 1-Step RT Mix	10 µL	80 µL	320 µL	-25°C to -15°C <sup>[4]</sup>
Power SYBR <sup>®</sup> Green 1-Step qRT-PCR Mix	500 µL	5 mL	20 mL	-25°C to -15°C Protect from light <sup>[5]</sup>

[1] 50-µL lysis reactions and 20-µL RT-PCR reactions

[2] Reported as “4°C” on the component label.

[3] Reported as “-20°C” on the component label.

[4] Keep *Power* SYBR<sup>®</sup> Green 1-Step RT Mix at -20°C until use; it can be stored on ice for short periods of time.

[5] After *Power* SYBR<sup>®</sup> Green 1-Step qRT-PCR Mix is thawed for the first time, it can be stored at 4°C. Protect from light when not in use.



## Required materials not provided with the kit

Unless otherwise specified, all materials are available from Life Technologies ([www.lifetechnologies.com](http://www.lifetechnologies.com)). MLS: Fisher Scientific ([www.fisherscientific.com](http://www.fisherscientific.com)) or other major laboratory supplier.

Item	Source
Real-time PCR instrument: <ul style="list-style-type: none"> <li>• Applied Biosystems® 7500 Real-Time PCR System</li> <li>• Applied Biosystems® 7500 Fast Real-Time PCR System (standard cycling only)</li> <li>• Applied Biosystems® 7900HT Fast Real-Time PCR System (standard cycling only)</li> <li>• StepOne™ Real-Time PCR System</li> <li>• StepOnePlus™ Real-Time PCR System</li> <li>• ViiA™ 7 Real-Time PCR System</li> <li>• QuantStudio™ Flex Systems</li> </ul>	Contact your local or regional sales representative.
Tubes, plates, seals, and other accessories required by the instrument	Visit <a href="http://www.lifetechnologies.com">www.lifetechnologies.com</a> , and select <b>Life Sciences ▶ PCR ▶ PCR Tubes, Plates &amp; Accessories</b>
Laboratory mixer (Vortex mixer or equivalent)	MLS
(If needed) Benchtop centrifuge, for washing suspension cells	MLS
Microcentrifuge	MLS
Adjustable micropipettors, 10 µL to 1000 µL	MLS
Aerosol-resistant pipette tips	MLS
Nuclease-Free Water	Cat. no. AM9938
10X PBS <sup>[1]</sup>	Cat. nos. AM9624, AM9625
PCR primers for the target of interest	User-supplied; see Appendix B, “Guidelines for custom-designed assays”
[Optional] SYBR® Green Cells-to-C <sub>T</sub> ™ Control Kit	Cat. no. 4402959

<sup>[1]</sup> Dilute to 1X with Nuclease-Free Water before use.

## Procedure overview

First, 10 to 10<sup>5</sup> cultured cells are washed with phosphate-buffered saline (PBS), then mixed with Lysis Solution and incubated at room temperature for 5 minutes. Cells are lysed during this incubation and RNA is released into the Lysis Solution, which contains reagents to inactivate endogenous RNases. If DNase I is added to the Lysis Solution (optional), genomic DNA is also degraded at this step.



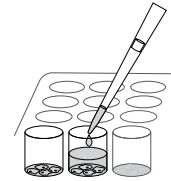
Next, Stop Solution is mixed into the lysate to inactivate the lysis reagents so that they will not inhibit the reverse transcription or PCR reactions. The resulting lysate is then used in one-step real-time RT-PCR with primers for the target of interest.

## Workflow

### Prepare cells for lysis

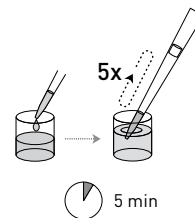
Adherent cells in 96- or 384-well plates: rinse with 1X PBS, then aspirate PBS.

Cells grown in other vessels (adherent or suspension): wash cells in 1X PBS, resuspend in 1X PBS at  $10^4$  to  $10^5$  cells per 5  $\mu$ L, and transfer 5  $\mu$ L to a 96-well plate.

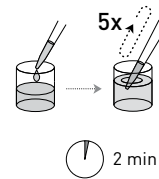


### Prepare the Cells-to- $C_T$ ™ lysate

Add 50  $\mu$ L of Lysis Solution to each well, pipette 5 times, and incubate at room temp. for 5 min.



Add 5  $\mu$ L of Stop Solution, pipette 5 times, and incubate at room temp for 2 min.



### Perform 1-step RT-PCR

Combine *PowerSYBR*® Green 1-Step qRT-PCR Mix and *PowerSYBR*® Green 1-Step RT Mix with primers and lysate, set up the instrument, then run the reaction.







## Important procedural guidelines

### Cells-to- $C_T$ <sup>™</sup> lysate preparation

- The lysis procedure is optimized for lysis of 10 to 10<sup>5</sup> cells per reaction.
- Do not vortex Stop Solution.
- Lysis Solution and Stop Solution must be at room temperature before starting the lysis procedure.
- We recommend storing Stop Solution in several tubes or plate wells (96-well format), for faster thawing and to reduce the number of freeze-thaw cycles.
- Avoid creating bubbles in the lysis procedure.  
For example, when mixing in Lysis Solution or Stop Solution, set the pipettor to ~35  $\mu$ L to pipet the ~50- $\mu$ L reactions.
- DNase treatment is recommended if primers do not span an exon junction.
- The minimum incubation time after addition of Stop Solution is 2 minutes.  
The incubation time can be extended to accommodate handling a large number of samples, but do not allow the reactions to remain at room temperature longer than 20 minutes after addition of Stop Solution.

### RT-PCR setup

- Cell type is the most significant factor in determining the optimal input lysate amount for the RT-PCR reaction.  
We recommend performing an optimization experiment when using the Cells-to- $C_T$ <sup>™</sup> 1-Step *Power* SYBR<sup>®</sup> Green Kit for the first time with a cell type.
  - Vary the number of cells in the lysis reaction between 10 and 10<sup>5</sup> cells.
  - Vary the volume of the lysate in the RT-PCR between 2% and 10% of the total RT-PCR reaction volume.
- The volume of the lysate should be between 2% and 10% of the total RT-PCR reaction volume; for most cell types, good results are seen with a lysate volume of 5%.  
Do not exceed:
  - 1600 cell equivalents for 20- $\mu$ L reactions.
  - 4000 cell equivalents for 50- $\mu$ L reactions.
- Do not pipette less than 1  $\mu$ L of lysate.  
If less than 1  $\mu$ L of lysate is required, dilute a portion of the lysate with Nuclease-Free Water, to pipette a larger volume, and reduce the volume of water in the RT-PCR reaction accordingly.
- The final concentration of forward and reverse PCR primers in the RT-PCR should be 100–200 nM.



- Include a no-template control (NTC) reaction: Use Nuclease-Free Water instead of lysate in the RT-PCR.  
This control tests for DNA contamination in the reagents and formation of primer-dimers.
- Include an appropriate number of PCR replicates or biological replicates, as required by your experimental needs.
- Follow “Good laboratory practices for PCR and RT-PCR” on page 17.

## Before each use of the kit

- Chill 1X PBS on ice, sufficient for 50  $\mu$ L per  $10^5$  cells.
- Thaw Stop Solution, with gentle mixing (*do not vortex*).  
Ensure that Stop Solution is at room temperature before use.
- (Optional) If DNase treatment is desired, prepare DNase/Lysis Solution master mix for the number of reactions required plus 10% overage. Prepare the master mix just before use.

Component	Volume per reaction
Lysis Solution (room temperature)	49.5 $\mu$ L
DNase	0.5 $\mu$ L

## Prepare cells for lysis

Prepare adherent or suspension cells for lysis.

Cell type	To prepare cells for lysis
Adherent cells grown in 96- or 384-well plates	<p>Use cells that have been cultured until they are fully adherent to the plate, to avoid losing cells during the wash.</p> <ol style="list-style-type: none"> <li>1. Aspirate culture medium from the wells and rinse with 50 <math>\mu</math>L of cold 1X PBS.</li> <li>2. Aspirate as much PBS as possible without disturbing the cells and proceed to “Prepare the Cells-to-CT lysate” on page 11.</li> </ol>
Cells grown in other vessels, including adherent and suspension cells	<ol style="list-style-type: none"> <li>1. Detach adherent cells from the culture vessel, using a common subculturing technique such as trypsin. If trypsin is used, be sure to inactivate it before proceeding.</li> <li>2. Count the cells, then gently centrifuge to pellet the cells.</li> <li>3. Discard the growth medium and resuspend the pellet in ~50 <math>\mu</math>L of chilled 1X PBS per <math>10^5</math> cells.</li> <li>4. Gently pellet the cells, and aspirate as much PBS as possible without disturbing the pellet.</li> <li>5. Resuspend in 5 <math>\mu</math>L of cold 1X PBS per 10 to <math>10^5</math> cells, and pipette up and down to thoroughly resuspend the cells.</li> <li>6. Distribute 5 <math>\mu</math>L of cells to the desired number of wells of a 96-well PCR plate and proceed to “Prepare the Cells-to-CT lysate” on page 11.</li> </ol>



## Prepare the Cells-to-C<sub>T</sub><sup>™</sup> lysate

1. Add 50 µL of room-temperature Lysis Solution or DNase/Lysis Solution to the prepared cells, and pipette up and down 5 times to mix well.
2. Incubate at room temperature for 5 minutes.
3. Add 5 µL of room-temperature Stop Solution and pipette up and down 5 times.
  - If you are using a multi-channel pipette, Stop Solution can be added directly to the lysis reaction.
  - If you are using a repeater pipette, Stop Solution can be added to the side wall of the well, but it is important to rinse the wall when pipetting up and down, to ensure thorough mixing of all of the Stop Solution with the lysis reaction.
4. Incubate at room temperature for 2 minutes.
5. Place the lysates on ice, and proceed to RT-PCR.

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**STOPPING POINT** Lysates can be stored on ice for up to 2 hours or at or below -20°C for up to 5 months.

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## Perform 1-step RT-PCR

1. Thaw all reagents, including previously frozen Cells-to-C<sub>T</sub><sup>™</sup> lysates, on ice.
2. On ice, prepare an RT-PCR Master Mix for the number of reactions required plus 10% overage.  
Adjust the final volume of RT-PCR Master Mix for the amount of lysate in each reaction.

**Table 2** RT-PCR Master Mix (for 20-µL reactions)

Component	Volume per 20-µL reaction <sup>[1]</sup>
PowerSYBR <sup>®</sup> Green 1-Step qRT-PCR Mix	10 µL
PowerSYBR <sup>®</sup> Green 1-Step RT Mix	0.16 µL
Gene-specific primer pool (100–200 nM final each primer)	variable
Nuclease-Free Water	To 19 µL (for 1 µL of lysate) To 18 µL (for 2 µL of lysate)

<sup>[1]</sup> Component volumes can be scaled for RT-PCR reaction volumes between 10 µL and 50 µL.

3. On ice, add the appropriate volume (18–19 µL) of RT-PCR Master Mix to each sample or NTC well of an optical reaction plate.
4. Add the appropriate volume (1–2 µL) of lysate or Nuclease-Free Water (for the NTC) to each well (20 µL total).



5. Seal the plate with an optical adhesive cover, vortex the plate for 5–10 seconds, then briefly centrifuge the plate.
6. Set up the real-time PCR instrument as indicated in the following table, then load and run the reactions.

Run the reactions shortly after setting them up; excessive exposure to light may affect the fluorescent dyes in *Power SYBR*<sup>®</sup> Green 1-Step qRT-PCR Mix.

**Table 3** Standard cycling conditions

Step	No. of cycles	Temp.	Time
Reverse transcription	1	48°C	30 min
Polymerase activation	1	95°C	10 min
Amplification	40	95°C	15 sec
		60°C	1 min
Melt curve <sup>[1]</sup> (optional)	1	95°C	15 sec
		60°C	15 sec
		95°C	15 sec

<sup>[1]</sup> A melt curve (dissociation curve) cycle can be run after the real-time RT-PCR, to detect non-specific amplification or primer dimers.



# Troubleshooting

For general real-time RT-PCR troubleshooting, refer to the appropriate guide for your instrument, or visit [www.lifetechnologies.com](http://www.lifetechnologies.com) and select **Life Sciences**  
 ▶ PCR ▶ Real-Time PCR (qPCR) ▶ qPCR Education.

## Cells-to-C<sub>T</sub><sup>™</sup> troubleshooting

Observation	Possible cause	Recommended action
No signal or unexpected signal, due to RT-PCR inhibition  The presence of RT-PCR inhibitors in the lysate has been confirmed after following "Perform 1-step RT-PCR with Xeno RNA Control" on page 15.	Components in the Lysis Solution are not fully inactivated by Stop Solution.	Add Stop Solution directly to the lysate: touch the lysate with the opening of the pipet tip when adding Stop Solution to ensure that the entire 5 µL of Stop Solution is added to each sample.
		Mix Stop Solution with the lysate by pipetting up and down 5 times.
	Too many cells were used in the lysis reaction, therefore RNase in the sample was not completely inactivated and/or cellular components or debris inhibited the RT-PCR.	Repeat the entire procedure (lysis and RT-PCR) with fewer cells (for example, 5- to 10-fold fewer cells).
		Perform an optimization experiment to determine the optimal number of cells for your cell type (see "RT-PCR setup" on page 9).
	Too much PBS (>5 µL) was left on the cells, diluting the Lysis Solution, therefore RNase in the sample was not completely inactivated.	Remove as much PBS as possible before adding Lysis Solution to the cells.
		If you split the cells after the PBS wash, resuspend cells in ≤5 µL of PBS for each sample of 10 to 10 <sup>5</sup> cells.



Observation	Possible cause	Recommended action
No signal or unexpected signal	RNA was degraded before starting the procedure.	To avoid RNA degradation, keep cells in PBS on ice before starting the lysis procedure. Take cells off ice just before adding Lysis Solution.
	Lysis Solution was not at room temperature, therefore cell lysis was not efficient.	Ensure that Lysis Solution is at room temperature before starting the lysis procedure.
	Lysates sat too long at room temperature before the RT-PCR reaction.	Do not allow lysates to sit longer than 20 minutes at room temperature once the Stop Solution has been added. If the RT-PCR reactions cannot be set up within this time: <ul style="list-style-type: none"> <li>• Store the lysates on ice for up to 2 hours after lysis.</li> <li>• Freeze the lysates at or below -20°C.</li> </ul>
	The sample does not contain the target RNA.	Before concluding that the sample does not contain the RNA of interest: <ul style="list-style-type: none"> <li>• Follow “Perform 1-step RT-PCR with Xeno RNA Control” on page 15 to determine whether the lysate contains RT-PCR inhibitors, but also perform parallel real-time RT-PCR reactions with primers for the target of interest. If PCR product is detected in the Xeno<sup>™</sup> RNA Control reaction but not in the RT-PCR for the RNA of interest, then it is possible that the RNA of interest is not expressed in the cells or is undetectable with this procedure.</li> <li>• Perform RT-PCR using purified RNA from the same cell culture source, to confirm that the PCR reagents and equipment work for the target of interest.</li> </ul>
Signal in the no-template control (NTC)	DNA contamination of reagents, pipettors, or benchtops.	Repeat the procedure with fresh reagents. Follow “Good laboratory practices for PCR and RT-PCR” on page 17.
	Primer-dimer amplification products, generated from amplification of self-annealed PCR primers.	Check the dissociation curve of NTC reactions: primer-dimer products are shorter than the expected amplicon, and thus will have a lower T <sub>m</sub> .



Observation	Possible cause	Recommended action
Unexpected signal  Melt curve analysis indicates amplification of the target, rather than amplification of primer-dimers.	Genomic DNA (gDNA) amplification.	Thoroughly mix DNase I with Lysis Solution.
		Use fewer cells per lysis reaction.
		Use Lysis Solution that is at room temperature, and make sure that the lysis reactions occur at room temperature (19–25°C).
		Use PCR primers designed to span an exon-exon boundary; amplicons from gDNA would thus be too long for efficient amplification.
		Perform a no-reverse transcription (no-RT) control, to determine whether gDNA is present in the lysate ("Perform a no-RT control experiment" on page 16). If PCR products are still seen in the no-RT control, try the following suggestions in the order shown: <ul style="list-style-type: none"> <li>• Increase the lysis reaction incubation time to 8 minutes.</li> <li>• Use Lysis Solution that has been warmed to 25°C.</li> </ul>

## Perform 1-step RT-PCR with Xeno™ RNA Control

Xeno™ RNA Control is a synthetic RNA transcript that lacks homology to current annotated biological sequences.

In this experiment, Xeno™ RNA Control and SYBR® Xeno™ Primers from the SYBR® Green Cells-to-C<sub>T</sub>™ Control Kit are used for RT-PCR in the presence and absence of lysate. If signal for Xeno™ RNA Control is detected only in reactions that do not contain lysate, or if reactions with lysate display a significantly higher C<sub>T</sub> (lower signal) than reactions without lysate, this indicates that RT-PCR inhibitors are present in the lysate.

The SYBR® Green Cells-to-C<sub>T</sub>™ Control Kit also includes primers for the endogenous control gene β-Actin (ACTB), providing an endogenous control for sample normalization (see "**Don't Bother Quantitating RNA for Gene Expression Analysis**" at [www.lifetechnologies.com](http://www.lifetechnologies.com)). Refer to the product information sheet for the SYBR® Green Cells-to-C<sub>T</sub>™ Control Kit (Pub. no. 4404891) for other applications of the kit.

1. Follow the main procedure, but prepare an RT-PCR Master Mix that includes Xeno™ RNA Control and primers instead of gene-specific primers, for the number of reactions plus 10% overage.

Prepare the master mix to accommodate the same volume of lysate that is used in RT-PCRs for the experimental samples.

**Table 4** RT-PCR Master Mix with Xeno™ RNA Control (for 20- $\mu$ L reactions)

Component	Volume per 20- $\mu$ L reaction
PowerSYBR® Green 1-Step qRT-PCR Mix	10 $\mu$ L
PowerSYBR® Green 1-Step RT Mix	0.16 $\mu$ L
20X SYBR® Xeno™ Primers <sup>[1]</sup>	1 $\mu$ L
Xeno™ RNA Control	0.036 $\mu$ L <sup>[2]</sup>
Nuclease-Free Water	To 19 $\mu$ L (for 1 $\mu$ L of lysate) To 18 $\mu$ L (for 2 $\mu$ L of lysate)

<sup>[1]</sup> 200 nM final concentration

<sup>[2]</sup> Use 3.6  $\mu$ L of Xeno™ RNA Control per 100 20- $\mu$ L reactions. Dilute Xeno™ RNA Control in Nuclease-Free Water for fewer reactions.

2. Add the appropriate volume (1–2  $\mu$ L) of lysate or Nuclease-Free Water (for the NTC) to each well.
3. Continue the RT-PCR procedure as for the experimental samples.

## Perform a no-RT control experiment

In this experiment, Power SYBR® Green 1-Step RT Mix (required for reverse transcription) is omitted from the reaction. If amplification of the target is seen in the absence of reverse transcription, this indicates that amplification of genomic DNA (gDNA) is occurring.

1. Follow the main procedure, but prepare no-RT reactions instead, using the following no-RT Master Mix for the number of replicates required plus 10% overage.

**Table 5** No-RT Master Mix

Component	Volume per 20- $\mu$ L reaction
PowerSYBR® Green 1-Step qRT-PCR Mix	10 $\mu$ L
Gene-specific primer pool (100–200 nM final each primer)	variable
Nuclease-Free Water	To 19 $\mu$ L (for 1 $\mu$ L of lysate) To 18 $\mu$ L (for 2 $\mu$ L of lysate)

2. Add the appropriate volume (1–2  $\mu$ L) of lysate or Nuclease-Free Water (for the NTC) to each well.  
Use the same volume of lysate that is used in RT-PCRs for the experimental samples.
3. Continue the RT-PCR procedure as for the experimental samples.



## Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. no. AM9890).



# Guidelines for custom-designed assays

We recommend using Primer Express<sup>®</sup> Software (Cat. no. 4363991) to select an amplicon site within the target of interest as well as the PCR primers for amplification. Refer to the *Primer Express<sup>®</sup> Software Version 3.0 Getting Started Guide* (Pub. no. 4362460) and the software online help.

## Guidelines for selecting the target amplicon site

Good amplicon site selection ensures amplification of cDNA from the target mRNA without co-amplification of its genomic sequence, pseudogenes, or cDNA from related genes. Following are general guidelines for choosing amplicon sites:

- The amplicon should span one or more introns, to avoid amplification of the target gene in genomic DNA.
- The primer pair must be specific to the target gene; the primer pair must not amplify pseudogenes or other related genes.
- Design primers following Primer Express<sup>®</sup> Software guidelines.
- Test the primer pairs, then select the primer pair that produces the highest signal-to-noise ratio (that is, earliest  $C_T$  with total RNA or mRNA and no amplification with genomic DNA or negative controls).
- If the gene of interest does not have introns, then it is not possible to choose an amplicon that will discriminate between cDNA and genomic DNA templates. For such targets, it is a good idea to include no-RT controls.

## Guidelines for primer design

- Keep the  $T_m$  between 58°C to 60°C.
- The optimal primer length is 20 bases.
- Keep the GC content in the 30 to 80% range.
- Avoid runs of identical nucleotides. If repeats cannot be avoided, there must be fewer than four consecutive G bases.
- Make sure the last 5 nucleotides at the 3' end contain no more than two G and/or C bases.
- If you cannot find acceptable primer sequences, you may need to examine the sequence and select another amplicon site or screen for more sites.

# Safety



**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
  - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
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## Chemical safety

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**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the “Documentation and Support” section in this document.
  - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
  - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
  - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
  - Handle chemical wastes in a fume hood.
  - Ensure use of 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.)
  - After emptying a waste container, seal it with the cap provided.
  - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used 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.
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## Biological hazard safety



**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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:  
[www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf](http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf)
  - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)
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# Documentation and support

## Obtaining SDSs

Safety Data Sheets (SDSs) are available from [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

**Note:** For SDSs of chemicals from third-party manufacturers, contact the chemical manufacturer.

## Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) and search for the Certificate of Analysis by product lot number, which is printed on the box.

## Obtaining support

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

[www.lifetechnologies.com/support](http://www.lifetechnologies.com/support)

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## Limited product warranty

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[lifetechnologies.com](http://lifetechnologies.com)

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