

TaqMan® Gold RT-PCR Kit

- Individual components provide flexibility in assay set-up to perform both one- and two-step RT-PCR.
- Random hexamers, oligo (dT)₁₆, and sequence-specific reverse primers provide flexibility for cDNA synthesis
- Optimized with high-quality enzymes to increase yield and provide robust results
- For the two-step protocol, AmpErase® UNG protects against carry-over contamination
- Includes Passive Reference I for accurate results and minimal well-to-well variability

Ideal for Gene Expression Experiments

The TaqMan® Gold RT-PCR Kit is ideal for studies involving detection and quantification of RNA targets. The reverse transcription (RT) step is performed at 48°C using MultiScribe™ Reverse Transcriptase. The amplification is performed with AmpliTaq Gold® DNA Polymerase.

Because of the modular nature of the kit, you can purchase additional RT or PCR modules as necessary. The complete kit provides enough reagents to perform 200 reactions.

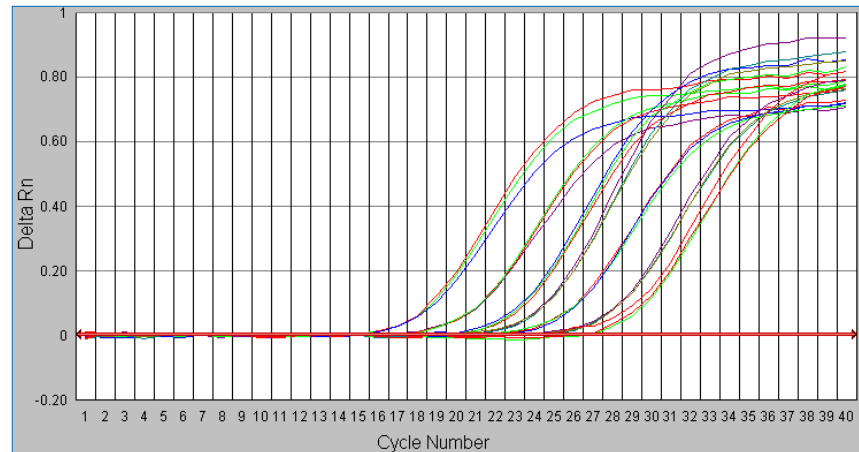


Figure 1. Two-step quantitative RT-PCR reaction using the TaqMan® Gold RT-PCR Kit. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was detected in total RNA samples (n=3) ranging from 100 ng to 256 fg on the ABI PRISM® 7000 Sequence Detection System.

The TaqMan® GAPDH control module (part of the kit) is sufficient for 100 control reactions. As with all TaqMan reagents, Passive Reference 1 is already included in the 10X TaqMan® Buffer A.

Flexible Quantitative RT-PCR

Because all components are provided separately, the TaqMan Gold RT-PCR Kit is flexible. Separate protocols let you perform one-step or two-step reactions (Figure 2).

Two-Step Reaction

The two-step reaction separates the RT step from the PCR step. The RT (typically performed in a 10 µL reaction) is performed first. When the RT is complete, the PCR components can be added to the same tube for amplification. Because

the reactions are separate, the RT and PCR steps may be optimized independently. Also, a portion of the cDNA generated by the RT step can be archived for other experiments.

We recommend the two-step reaction for assays designed to examine the expression of multiple different transcripts from the same reverse transcription reaction.

The reverse transcription is typically performed using random hexamers or oligo (dT)₁₆. The subsequent PCR amplifications are performed using different gene-specific primers.

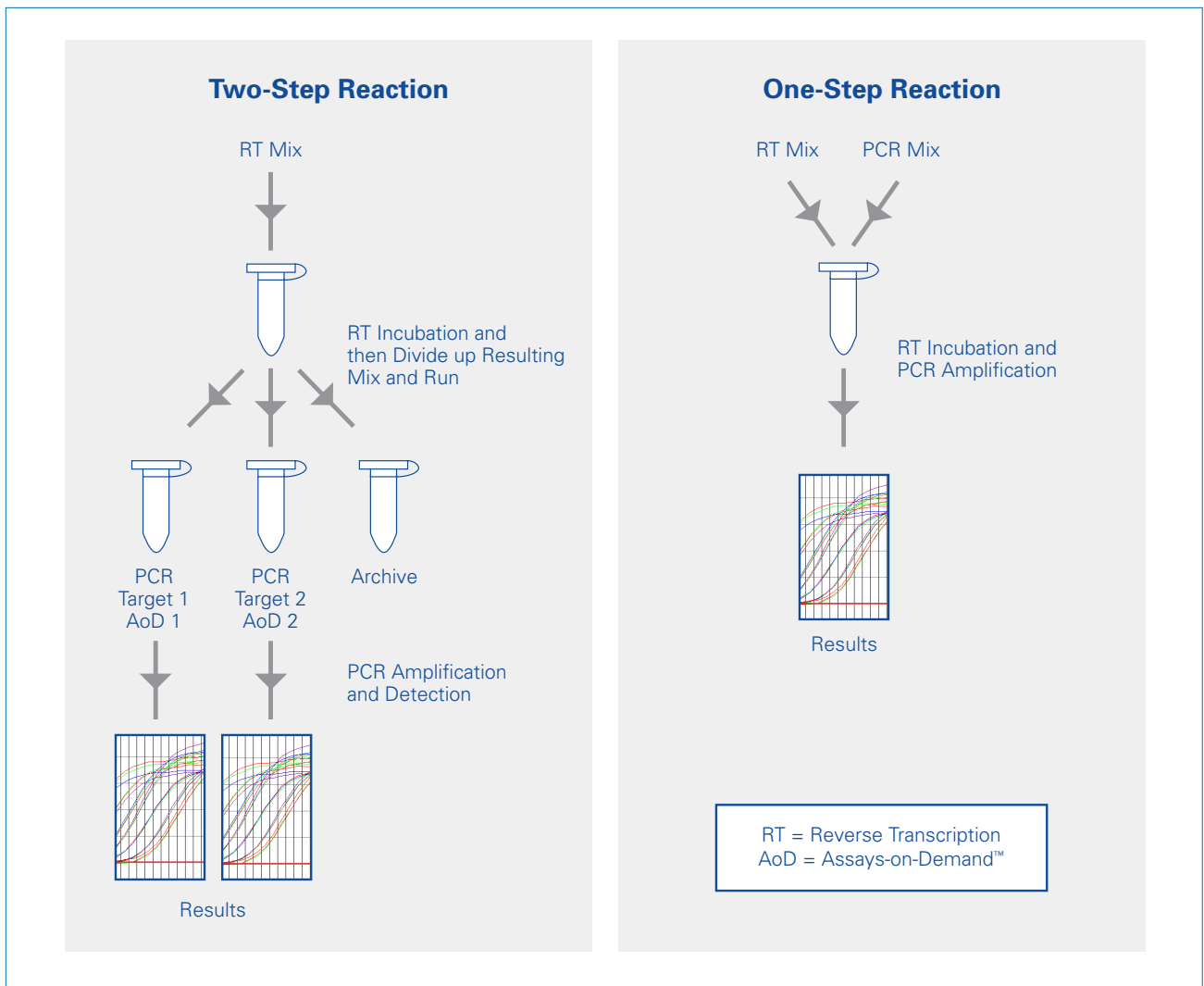


Figure 2. Comparison of Two-Step and One-Step RT-PCR Reactions

One-Step Reaction

The one-step feature lets you set up both RT and PCR reactions at the same time in the same tube. When you are investigating the expression of a single transcript, the one-step procedure helps you maximize convenience and throughput.

High Quality Enzymes

Whether you run one-step or two-step reactions, the MultiScribe Reverse Transcriptase and AmpliTaq Gold DNA Polymerase combination give you consistently robust results.

MultiScribe Reverse Transcriptase is Moloney Murine Leukemia Virus (MuLV) Reverse Transcriptase that has been optimized for TaqMan-based assays. The recommended use of MultiScribe Reverse Transcriptase is different from the recommended use of MuLV as detailed in GeneAmp® kits. AmpliTaq Gold DNA Polymerase is a hot-start Taq enzyme that provides higher yield, better specificity, and more reliable results.

Internal Passive Reference

The 10X TaqMan® Buffer A contains an internal passive reference to provide more accurate results and minimize well-to-well variability. This buffer is included in the TaqMan® PCR Core Reagents Kit, which is a module of the TaqMan Gold RT-PCR Kit.

Contamination Control

It is important to note that contamination control using AmpErase® UNG cannot be used with the one-step procedure of the TaqMan Gold

RT-PCR Kit, because the procedure uses a 48°C reverse transcription temperature. If you desire a one-step reaction with contamination control, we recommend the TaqMan® EZ RT-PCR Kit.

	TaqMan® 1-Step RT-PCR Master Mix	TaqMan® Gold RT-PCR Kit	TaqMan® EZ RT PCR Kit
Kit purpose	General Kit	General Kit	High Temperature RT Kit
Kit format	Master Mix	Separate components (Core reagent)	Separate components (Core reagent)
RT enzyme	MultiScribe™ Reverse Transcriptase	MultiScribe™ Reverse Transcriptase	rTth DNA Polymerase
PCR enzyme	AmpliTaq Gold® DNA Polymerase	AmpliTaq Gold® DNA Polymerase	rTth DNA Polymerase
T _m	Low (48°C)	Low (48°C)	High (60°C)
RT Primers	Gene-specific primers	-Gene-specific primers -Random hexamers -Oligo dT	Gene-specific primers only (T _m minimum of 60°C)
Number of steps	One-step	One-step or two-step (flexible)	One-step
AmpErase® UNG compatible	No	Yes (two-step only)	Yes
RT minus controls	Yes	Yes	Substitute Mn ²⁺
Recommended use	High-throughput	-Various assays to develop -Detect multiple targets from one cDNA	-Few assays to develop -High-temperature RT would benefit assay performance
Application	Gene expression studies	Gene expression studies	Targets with high degree of secondary structure (i.e., viral targets)

Minimal Reaction Optimization

Primers and probes used in this assay will prove most effective when you follow guidelines for TaqMan® probe and primer design. These guidelines are detailed in the TaqMan® Gold RT-PCR Kit Protocol. The result will be faster assay development and minimal optimization.

Demonstrated Performance

The TaqMan® Gold RT-PCR kit reagents and protocol produce a demonstrated detection range for human GAPDH mRNA of between 2 picograms and 200 nanograms of total RNA control, which is approximately 200 to 2×10^7 transcript copies.

Ordering Information

Description	P/N
TaqMan® Gold RT-PCR Kit, with controls	N808-0233
TaqMan® Gold RT-PCR Kit, without controls	N808-0232
TaqMan® Reverse Transcription Reagents	N808-0234
TaqMan® GAPDH Control Reagents	402869
TaqMan® Gold RT-PCR Kit Protocol	402876
10-pack, TaqMan® Gold RT-PCR Kit, without controls	430413



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