

TaqMan[®] Gold RT PCR Kit



- Robust reaction chemistry
- Flexible procedure-1 or 2 step reactions
- Multiscribe[™] Reverse Transcriptase and AmpliTaq Gold[™] DNA Polymerase

Ideal for Gene Expression Experiments

The TaqMan[®] Gold RT-PCR Kit is ideal for studies involving detection and quantitation 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.

The modular nature of the kit enables RT or PCR modules to be purchased as necessary. The complete kit provides enough reagents to perform 200 reactions of 50 lL each. 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 contained in the 10X TaqMan[®] Buffer A.

Two-Step Reaction Chemistry

The TaqMan Gold RT-PCR Kit is a flexible kit. Protocols are provided to perform one-step or two-step reactions (Figure 1). The two-step reaction chemistry separates the RT step from the PCR step. Because the reactions are separate they may be optimized independently.

The two-step reaction chemistry is recommended 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. The subsequent PCR amplifications are performed using different gene-specific primers (Figure 2).

One-Step Reaction Chemistry

A one-step reaction chemistry enables both RT and PCR reactions to be set up at the same time in the same tube. When looking at the expression of a single transcript, the one-step procedure helps to maximize convenience and throughput.



Two Enzyme System

Whether doing one-step or two-step reaction chemistry, the Multiscribe Reverse Transcriptase and AmpliTaq Gold DNA Polymerase combination provide consistently robust results.

Multiscribe Reverse Transcriptase is Moloney Murine Leukemia Virus (MuLV) Reverse Transcriptase that has been optimized for TaqMan assays. The recommended usage of Multiscribe Reverse Transcriptase is different than the recommended usage of MuLV as detailed in GeneAmp[®] kits. AmpliTaq Gold DNA Polymerase is a high performance enzyme providing 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 (N808-0228), 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, due to the use of a 48 °C reverse transcription temperature. If a one-step reaction with contamination control is desired, the TaqMan[®] EZ RT-PCR Kit (P/N N808-0235) is recommended.

Two-Step Reaction Chemistry
Step 1. Set up FIT Reaction Mix

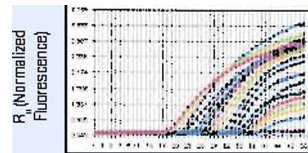


Step 2. Set up PCR Reaction Mix



Detection

Amplification Plot



One-Step Reaction Chemistry

Step 1. Set up RT and PCR Reaction Mix



Detection

Amplification Plot

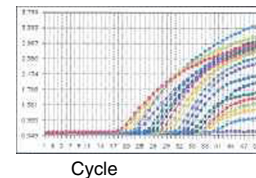


Figure 1. Comparison of Two-Step and One-Step Reaction Chemistry.

Choices for TaqMan RT-PCR

	TaqMan Gold RT-PCR Kit	TaqManEZ RT-PCR Kit
Kit Purpose	General Kit	High Temperature RT Kit
RT enzyme	MultiScribe™ Reverse Transcriptase	rTth DNA Polymerase
PCR enzyme	AmpliAq Gold™ DNA Polymerase	rTth DNA Polymerase
FIT temperature	Low (48 °C)	High (60 °C)
RT primers	<ul style="list-style-type: none"> Gene-specific primers Random hexamers Oligo dT 	<ul style="list-style-type: none"> Gene-specific primers only (Tm: minimum of 60 °C)
Number of steps	One-step or two-step (flexible)	One-step
AmpErase® UNG compatible	Yes (two-step only)	Yes
RT minus control	Yes	Substitute Mgt*
Recommended when...	<ul style="list-style-type: none"> Many different assays to develop Detect multiple different targets from one cDNA 	<ul style="list-style-type: none"> Few different assays to develop High-temperature RT would benefit assay performance
Application	Gene expression studies	Pathogen detection

Two-Step Reaction Chemistry
RT Control for Brain Samples

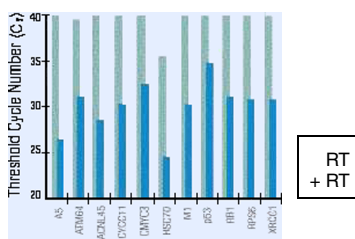


Figure 2. Multiple transcripts amplified using the two-step method. Eleven different transcripts were amplified from a single cDNA reaction of human brain total RNA using the two-step method with the TaqMan Gold RT-PCR Kit. Also indicated are control reactions where MultiScribe Reverse Transcriptase was added (+RT) or omitted (-RT). The transcripts from left to right are as follows: A5=(1-Actin; ATM64=Ataxia telangiectasia gene; CACNL45=a1A-voltage dependent calcium channel; CYCC11=Cyclin C; CMYC3=c-MYC; HSC70=Heat shock cognate; M1=Acetylcholine receptor; p53=p53; R81= Retinoblastoma gene; RPS6=Ribosomal protein S6; XRCC1=DNA repair enzyme.

SAMPLE FILE COPY

Minimal Reaction Optimization

Primers and probes used in this assay, with these reagents, will provide the greatest measure of success when guidelines for TaqMan probe and primer design are followed. These guidelines are detailed in the TaqMan® Gold RT-PCR Kit Protocol (P/N 402876). The result will be faster assay development and minimal optimization.

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Demonstrated Performance

Using the TaqMan Gold RT-PCR Kit reagents and protocol, the detection range for human GAPDH mRNA has been demonstrated to be between two picograms and 200 nanograms of total RNA control, which is approximately 200 to 2 x 10⁶ 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® PCR Core Reagent Kit	N808-0228
TaqMan® Gold RT-PCR Kit Protocol	402876
10-Pack TaqMan® Gold RT-PCR Kit, without controls	4304133
10-Pack TaqMan® Reverse Transcription Reagents	4304134

Applied Biosystems

Applied Biosystems POP reagents are developed and manufactured by Roche Molecular Systems, Inc. Branchburg, New Jersey, U.S.A.

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MultiScribe is a trademark and ABI Prism and its design, Applied Biosystems, and GeneAmp are registered trademarks of Applied Biosystems Corporation or its subsidiaries in the U.S. and certain other countries.

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and South Africa (Johannesburg)	27 11 478 0411	27 11 478 0349
Middle Eastern Countries and North Africa (Monza, Italy)	39 (0)39 8389 481	39 (0)39 8389 493
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	61 3 9730 8600	61 3 9730 8799
China (Beijing)	86 10 64106608	86 10 64106617
Hong Kong	852 2756 6928	852 2756 6968
Korea (Seoul)	82 2 593 6470/6471	82 2 593 6472
Malaysia (Petaling Jaya)	60 3 758 8268	60 3 754 9043
Singapore	65 896 2168	65 896 2147
Taiwan (Taipei Hsien)	886 2 2358 2838	886 2 2358 2839
Thailand (Bangkok)	66 2 719 6405	66 2 319 9788
Europe		
Austria (Wien)	43 (0)1 867 35 75 0	Fax 43 (0)1 867 35 75 11
Belgium	32 (0)2 712 5555	32 (0)2 712 5516
Czech Republic and Slovakia (Praha)	420 2 61 222 164	20 2 61 222 168
Denmark (Naerum)	45 45 58 60 00	45 45 58 60 01
Finland (Espoo)	358 (0)9 251 24 250	358 (0)9 251 24 243
France (Paris)	33 (0) 1 69 59 85 85	33 (0) 1 69 59 85 00
Germany (Weiterstadt)	49 (0) 6150 101 0	49 (0) 6150 101 101
Hungary (Budapest)	36 (0) 1 270 8398	36 (0) 1 270 8288
Italy (Milano)	39 (0)39 83891	39 (0)39 838 9492
Norway (Oslo)	47 23 12 06 05	47 23 12 05 75
Poland, Lithuania, Latvia, and Estonia (Warszawa)	48 (22) 866 40 10	48 (22) 866 40 20
Portugal (Lisbon)	351 (0)22 605 33 14	351 (0)22 605 33 15
Russia (Moskva)	7 095 935 8988	7 095 564 8787
South East Europe (Zagreb, Croatia)	385 1 34 91 927	385 1 34 91 840
Spain (Tres Cantos)	34 (0)91 806 1210	34 (0)91 806 1206
Sweden (Stockholm)	46 (0)8 619 4400	46 (0)8 619 4401
Switzerland (Rotkreuz)	41 (0)41 799 7777	41 (0)41 790 0676
The Netherlands (Nieuwerkerk a/d IJssel)	31 (0)180 331400	31 (0)180 331409
United Kingdom (Warrington, Cheshire)	44 (0)1925 825650	44 (0)1925 282502
All other countries not listed (Warrington, Cheshire)	44 (0)1925 282481	44 (0)1925 282509
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	81 3 5566 6230	81 3 5566 6507
Latin America		
Del.A. Obregon, Mexico	305-670-4350	305-670-4349
USA and Canada		
Internet	1-800-497-6809	support@appliedbiosystems.com
		www.appliedbiosystems.com/techsupport