TaqMan® Reverse Transcription Reagents

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Print Options

Package Contents

Catalog Number	Size
N8080234	200 rxns
4304134 (10-pack)	2000 rxns

Kit Contents

Storage Conditions

- Store all contents at –20°C.
- Reverse Transcriptase and RNase Inhibitor are sensitive to air oxidation.
- Protect the fluorescent-dye labeled probe from light.

Required Materials

- Template: RNA
- Reverse gene-specific primers
- DEPC-treated water (Cat. no. AM9916)
- *Optional:* 1 M DTT (Cat. no. P2325)

Additional Materials

Timing

Varies depending on primer used.

Selection Guides

Go online to view related products.
PCR Enzymes and Master Mixes
RT Enzymes and Kits
Real-Time PCR Instruments
Real-Time PCR Master Mixes

Product Description

- TaqMan® Reverse Transcription Reagents are designed for reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA. The reagents in this kit facilitate two-step RT-PCR.
- Two-step RT-PCR is performed in two separate reactions, useful when detecting multiple transcripts from a single cDNA reaction, or when storing a portion of the cDNA.
- TaqMan® Reverse Transcription Reagents contains MultiScribe™ Reverse Transcriptase and all other components needed for an RT set-up for 1st strand synthesis.
- MultiScribe™ Reverse Transcriptase is a recombinant Murine Leukemia Virus reverse transcriptase (MuLV RT).

Important Guidelines

- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- Dilute the 1M DTT stock to 100 mM in water prior to use in the RT reaction.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

Enzyme Characteristics:

Recombinant MuLV RT

RT Reaction Setup

Use the measurements below to prepare your RT reaction, or enter your own parameters in the column provided.

Component	20-µL rxn	Custom	Final Conc.
DEPC-treated water	to 20 µL	to µL	—
10X RT Buffer	2.0 µL	µL	1X
25 mM $MgCl_2$	1.4 µL	µL	1.75 mM
10 mM dNTP mix (2.5 mM each)	4.0 µL	µL	0.5 mM each
100 mM DTT* (optional)	1.0 µL	µL	5 mM
RNase Inhibitor (20 U/µL)	1.0 µL	µL	1.0 U/µL
MultiScribe™ RT (50 U/µL)	1.0 µL	µL	2.5 U/µL
50 µM Oligo $d(T)_{16}$, or 50 µM Random hexamers, or 10 µM gene-specific reverse primer	1.0 µL 1.0 µL 1.0 µL	µL	2.5 µM 2.5 µM 0.5 µM
Template RNA	varies	µL	< 1 µg/rxn

* Dilute the 1 M DTT stock to 100 mM in water prior to using in your RT reaction.

RT, PCR, and qPCR Protocols

View the procedures for preparing and running your RT, PCR, and qPCR experiments starting on page 2.

Optimization Strategies

Refer to the pop-ups below for guidelines to optimize your RT, PCR, and qPCR reactions.

RNA Sample Prep
RT Guidelines
PCR Guidelines
qPCR Guidelines

Limited Warranty, Disclaimer, and Licensing Information

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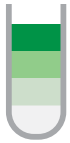
TaqMan® Reverse Transcription Reagents

The example RT procedure below shows reagent volumes for a single **20-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube prior to adding template RNA and primers. cDNA generated with this protocol will be used to perform subsequent PCR or qPCR.

1 Thaw reagents

Keep the enzymes in a freezer until immediately prior to use.
Thaw all reagents and keep them on ice. Mix and briefly centrifuge the components.

2 Prepare RT master mix



Combine the following components in each reaction tube.

Note: Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.

Note: The reaction volume for the RT step can be varied from 10 to 100 µL. A 100-µL RT reaction will efficiently convert a maximum of 2.0 µg total RNA to cDNA.

Component	20-µL rxn	Final Concentration
DEPC-treated water	to 20 µL	—
10X RT Buffer	2.0 µL	1X
25 mM $MgCl_2$	1.4 µL	1.75 mM
10 mM dNTP mix (2.5 mM each)	4.0 µL	0.5 mM each
100 mM DTT	1.0 µL	5.0 mM
RNase Inhibitor (20 U/µL)	1.0 µL	1.0 U/µL
MultiScribe™ RT (50 U/µL)	1.0 µL	2.5 U/µL

Cap, mix, and briefly centrifuge the components.

Note: If using oligo $d(T)_{16}$ or reverse gene specific primers, incubate RNA and primer following the first two cycling steps (highlighted below), then add master mix and continue with the next three cycling steps. For random hexamers, combine master mix, primer, and RNA, and then cycle as noted below.

3 Add template RNA and primers

Component	20-µL rxn	Final Concentration
50 µM Oligo $d(T)_{16}$, or 50 µM random hexamers, or 10 µM reverse gene-specific primer	1.0 µL 1.0 µL 1.0 µL	2.5 µM 2.5 µM 0.5 µM
Template RNA	varies	< 1 µg

Cap each tube, mix, and then briefly centrifuge the contents.

4 Incubate reactions in a thermal cycler

Follow the recommended cycling parameters based on your selected primer type for this experiment.

Primer Type	Temperature	Time
Oligo $d(T)_{16}$ or gene-specific primers	65°C	5 minutes
	4°C	2 minutes
	37°C	30 minutes
	95°C	5 minutes
	4°C	indefinitely
Random hexamers	25°C	10 minutes
	37°C	30 minutes
	95°C	5 minutes
	4°C	indefinitely

Use your RT reaction immediately for down-stream applications, or store it at –20°C.

PCR Protocol

The example PCR procedure below shows reagent volumes for a single **50-µL** reaction using AmpliTaq® DNA Polymerase with Buffer I, as well as the cDNA generated by following the RT protocol on page 2. For other DNA Polymerases, refer to their respective manufacturers' guidelines. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2– 0.5 mL PCR reaction tube prior to adding primers and template cDNA.

1 Thaw reagents

Thaw, mix, and briefly centrifuge each component before use.

Keep components on ice.

2 Prepare PCR master mix

Add the following components to appropriate wells or tubes.

Note: Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.

Component	50-µL rxn	Final Concentration
Autoclaved, distilled water	to 50 µL	—
10X PCR Buffer I*	5.0 µL	1X
10 mM dNTP mix (2.5 mM each)	1.0 µL	0.2 mM
AmpliTaq® DNA Polymerase (5 U/uL)	0.25 µL	0.025 U/µL

* Buffer I contains $MgCl_2$.

Mix and briefly centrifuge the components.

3 Add RT reaction cDNA and primers

Add your primers and template cDNA to each tube for a final reaction volume of 50 µL.

Component	50-µL rxn	Final Concentration
10 µM forward primer	1.0 µL	0.2 µM
10 µM reverse primer	1.0 µL	0.2 µM
RT reaction cDNA	varies	< 500 ng/rxn*

Cap each tube, mix, and then briefly centrifuge the contents.

4 Incubate reactions in a thermal cycler

Note: You can use two-step cycling (skipping the anneal step) when the primer T_m is > 50°C.

Step		Temperature	Time
Initial denaturation		95°C	2 minutes
35 PCR cycles	Denature	95°C	15 seconds
	Anneal	~55°C (depending on primer T_m)	30 seconds
	Extend	72°C	1 minute/kb
Final extension		72°C	5 minutes
Hold		4°C	indefinitely

5 Analyze with gel electrophoresis

Analyze 10 µL using agarose gel electrophoresis.

Use your PCR reaction immediately for down-stream applications, or store it at –20°C.

qPCR Protocol

The example PCR procedure below shows reagent volumes for a single **10-µL** reaction, using EXPRESS qPCR Supermix, Universal, as well as cDNA generated by following the RT protocol on page 2. For other pPCR mixes, refer to their respective manufacturers' guidelines.

For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube, or well of a MicroAmp® EnduraPlate™ Optical 96- or 384-well plate, prior to adding template DNA and primers.

	Steps	Procedure Details
1 	**Thaw reagents**	Thaw reagents. For 384-well plates, we recommend a maximum reaction volume of 10 µL per well. **Note:** Always prepare no-template control (NTC) reactions to test for DNA contamination of the enzyme/primer mixes.
2 	**Prepare qPCR master mix**	See table below
3 	**Incubate reactions in a real-time instrument**	See table below
4 	**Collect and analyze data**	Analyze results following your Real-Time instrument manufacturer's guidelines. *Optional:* The specificity of the PCR products can be checked by agarose gel electrophoresis.

Component	10-µL rxn	Final Concentration
Autoclaved, distilled water	to 10 µL	—
EXPRESS qPCR Supermix, Universal	5.0 µL	1X
20X Fluorescent Primer/Probe Mix	0.5 µL	1X
ROX Reference Dye (25 µM)	0.2 µL	0.5 µM
RT reaction cDNA	2.0 µL	varies

Cap or seal each PCR tube/plate, gently mix, and centrifuge contents.

Step		Temperature	Time
Pre-incubation		95°C	10 minutes
40 cycles	Amplification	95°C	15 seconds
		60°C	1 minutes