

One-Step RT-qPCR Kit (Dye-Based)

Catalog No.	50504DS	50504DL
Reactions	100 reactions	500 reactions

Description

One-Step RT-qPCR Kit (Dye-Based) is a fast and convenient solution for performing real-time PCR. The kit combines reverse transcription and qPCR into one reaction, which eliminates the need for two step reactions with the additional transfer samples between the reverse transcription and qPCR steps, thus minimizing sample handling and reducing the risk of contamination.

The proprietary buffer components facilitate the melting of secondary structures of nucleic acids and ensure ultimate complete cDNA synthesis and specificity of the PCR product in just 1.5 hours. The unique formulation of both buffer and enzyme mix delivers consistent performance, and reduces cost and setup time. A unique DNA intercalating dye is used to replace SYBR Green I, but with less PCR inhibition. The reference dye ROX is included for qPCR normalization when needed, making the kit compatible with all real-time PCR instruments.

One-Step RT-qPCR Kit has wide dynamic range, superior sensitivity, specificity, and reproducibility. The kit is ideal for both low-throughput and high-throughput real-time PCR screening and validation.

Features

- Fast and versatile RT-qPCR compatible with SYBR-Green based assays
- Efficient cDNA synthesis coupled with robust qPCR
- Superior sensitivity, specificity, and reproducibility
- Compatible with all real-time PCR instruments - reference dye ROX is included
- Unique buffer and enzyme formulation delivers consistent performance, saving costs and time.

Component

Catalog No.	50504DS	50504DL
RS Buffer (5X)	400 μ l	2000 μ l
RP Enzyme	100 μ l	500 μ l
ROX (25 μ M)	50 μ l	250 μ l
PCR Water	1500 μ l	7500 μ l

Storage Condition

- Store kit at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Real Time PCR thermal cycler
- qPCR plate
- qPCR plate seal film

Protocol

Reaction setup

Suggested reaction setup for SYBR-Green based assays (on ice recommended):

Component	Volume	Final conc.
RNA template*	variable	
RS Buffer (5X)	4.0 μ l	1X
Forward Primer (10 μ M)	0.8 μ l	0.4 μ M
Reverse Primer (10 μ M)	0.8 μ l	0.4 μ M
ROX (25 μ M)**	variable	
RP Enzyme	1.0 μ l	1X
Water	variable	
Total Volume	20 μl	

* RNA template between 1 μ g and 1 pg can be used. The amount can be varied dependent on the expression levels of the targeted genes.

** ROX may be required based on the model of the thermal cycler.

Reaction Condition

Suggested condition for RT-qPCR:

Step	Temperature	Time	Cycles
Reverse Transcription	42°C	20 minutes	1
Initial Denaturation	95°C	30 seconds	1
Denaturation	95°C	15 seconds	35-45 cycles
Annealing/Extension	60°C	30 seconds	
Melt Curve	60-95°C	variable	1

Note:

- For the cDNA synthesis of long transcripts, extend the Reverse Transcription step up to 45 min.
- Melt Curve: Follow instrument instruction if needed.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.




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