BIO-RAD

iScript™ Reverse Transcription Supermix for RT-qPCR

Catalog #	Description
1708840	**iScript Reverse Transcription Supermix for RT-qPCR**, 100 µl of 5x supermix, 25 reactions
1708841	**iScript Reverse Transcription Supermix for RT-qPCR**, 4 x 100 µl of 5x supermix, 100 reactions

For research purposes only.

Introduction

The iScript Reverse Transcription Supermix for RT-qPCR (iScript RT Supermix) is a sensitive, fast, and convenient reagent for gene expression analysis using real-time reverse transcription quantitative PCR (RT-qPCR) and standard RT-PCR. The preblended 5x supermix contains in one tube all the necessary components, except RNA template, for first-strand cDNA synthesis.

- **Simple and fast** — short protocol time (40 min) and 1-tube master mix format allow easy setup and fast qPCR results
- **Data reproducibility** — 1-tube supermix format reduces pipetting steps and promotes consistent and reproducible results
- **Broad dynamic range** — works with a broad linear dynamic range of input total RNA (1 µg–1 pg) and allows sensitive detection of target genes with low expression levels
- **Primer design flexibility** — includes an optimum blend of oligo(dT) and random primers to provide unbiased representation of the 5' and 3' regions of target genes for freedom in qPCR primer design

Storage and Stability

Store at –20°C. Guaranteed for 12 months at –20°C in a constant temperature freezer (this reagent will not freeze at –20°C).

Kit Contents

Reagent	Description
iScript RT Supermix (gray cap, 25 or 100 reactions)	5x RT supermix with RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase, RNase inhibitor, dNTPs, oligo(dT), random primers, buffer, $MgCl_2$, and stabilizers
iScript No-RT Control Supermix (clear cap, 50 reactions)	5x no-RT control supermix formulated to serve as a no-enzyme control, contains all components of iScript RT Supermix except reverse transcriptase
Nuclease-free water	—

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Reaction Setups

Reaction Setup for a Single cDNA Synthesis Reaction

For optimal results, reactions should be assembled on ice using appropriate reaction vessels.

Component	Volume per Reaction, µl
iScript RT Supermix	4
RNA template (1 µg–1 pg total RNA)	Variable
Nuclease-free water	Variable
Total volume	**20**

Reaction Setup for Multiple cDNA Synthesis Reactions

The following table shows an example of a master mix preparation for ten reactions with 5 µl input RNA (1 µg–1 pg) and enough excess master mix to accommodate loss during pipetting.* For optimal results, reactions should be assembled on ice using appropriate reaction vessels.

Component	Volume per Reaction, µl
iScript RT Supermix	48
Nuclease-free water	132
Total volume	**180**

1. Prepare the reverse transcription master mix as indicated in the table above. Mix thoroughly by pipetting up and down several times.
2. Add 15 µl master mix to 5 µl RNA for each reverse transcription reaction.
3. Adjust the volume of water if the input RNA volume is not 5 µl input RNA (1 µg–1 pg), as stated in the example above.

* If more reactions are required, scale up appropriately. The volume of supermix provided in 25- and 100-reaction kits does not take into account the preparation of excess master mix.

Reaction Protocol

Incubate the complete reaction mix in a thermal cycler using the following protocol:

Priming	5 min at 25°C
Reverse transcription	20 min at 46°C
RT inactivation	1 min at 95°C

Recommendations for the Use of No-RT Control

- Interference of gene expression analysis by genomic DNA carryover in RNA samples can be tested using the no-RT control supermix
- Setting up a no-RT control reaction with the same amount of total RNA as the reverse transcription reaction is recommended to allow similar carryover of cDNA synthesis components in qPCR for accurate detection of genomic DNA amplicons

Recommendations for qPCR

- cDNA generated with this kit can be used directly in qPCR
- The volume of cDNA synthesis reaction used must not exceed 10% of the qPCR volume
- cDNA can be diluted in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA prior to use in qPCR. The optimum cDNA dilution must be determined based on target gene abundance and qPCR chemistry

Related Products

Catalog #	Description
Reverse Transcription Reagents for Real-Time qPCR	
1725037	iScript Advanced cDNA Synthesis Kit for RT-qPCR
1708890	iScript cDNA Synthesis Kit
1708896	iScript Select cDNA Synthesis Kit
1725034	iScript gDNA Clear cDNA Synthesis Kit
Reagents for Real-Time qPCR	
1725270	SsoAdvanced™ Universal SYBR® Green Supermix
1725280	SsoAdvanced Universal Probes Supermix
1725120	iTaq™ Universal SYBR® Green Supermix
1725130	iTaq Universal Probes Supermix
1725160	SsoAdvanced PreAmp Supermix

Visit **bio-rad.com/web/iscriptRTsmx** for more information.

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