BIO-RAD

iScript™ Advanced cDNA Synthesis Kit for RT-qPCR

Catalog #	Description
1725037	**iScript Advanced cDNA Synthesis Kit for RT-qPCR**, 25 x 20 µl reactions
1725038	**iScript Advanced cDNA Synthesis Kit for RT-qPCR**, 100 x 20 µl reactions

For research purposes only.

Introduction

The iScript Advanced cDNA Synthesis Kit for RT-qPCR is an enhanced formulation that offers increased data throughput from a single 20 µl reverse transcription (RT) reaction for real-time quantitative PCR (qPCR). This two-tube kit enables superior capacity as well as a wide linear dynamic range for reverse transcription.

- **Increase qPCR data throughput and cost effectiveness from a single reaction** — cDNA synthesized from higher input RNA allows the analysis of a large number of target genes
- **Reduce interassay variability** — higher yields of cDNA offer flexibility of qPCR replicates
- **Detect low-level target genes** — uncompromised sensitivity even with lower input RNA amounts, in which sample is limited

Storage and Stability

Store at –20°C. Guaranteed for 12 months at –20°C in a constant temperature freezer.

Kit Contents

Reagent	Description
5x iScript Advanced Reaction Mix	5x reaction mix with dNTPs, oligo(dT), and random primers
iScript Advanced Reverse Transcriptase	RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase and RNase inhibitor
Nuclease-free water	1.5 ml

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
5x iScript Advanced Reaction Mix	4
iScript Advanced Reverse Transcriptase	1
RNA template (100 fg–7.5 µg)*	Variable
Nuclease-free water	Variable
Total volume	**20**

* Input RNA amounts must be optimized based on target gene abundance and sample availability. Ensuring the quality and purity of the RNA sample is essential for achieving the highest capacity.

Reaction Setup for Multiple cDNA Synthesis Reactions

The example below shows a master mix preparation for ten reactions with 5 µl input RNA and enough excess master mix to accommodate loss during pipetting (in this case 12 reactions). For optimal results, reactions should be assembled on ice using appropriate reaction vessels.

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

Component	Volume per Reaction, µl
5x iScript Advanced Reaction Mix	48
iScript Advanced Reverse Transcriptase	12
Nuclease-free water	120
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 of the prepared master mix to 5 µl input total RNA for each reverse transcription reaction.
3. Adjust the volume of water if the input RNA volume differs from the above example. The final volume of master mix to be pipetted into each reaction should also be adjusted.

Reaction Protocol

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

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

Recommendations for qPCR

- For input RNA (1.0 µg–7.5 µg): cDNA generated with this kit must be diluted at least 10-fold in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, or nuclease-free water prior to use in qPCR
- For input RNA less than 1 µg: cDNA generated with this kit can be used directly in qPCR
- Optimum cDNA dilution must be determined based on target gene abundance and qPCR chemistry
- The volume of cDNA synthesis reaction used must not exceed 10% of the qPCR volume

Recommendations for the Use of No-RT Control

- Contamination by genomic DNA carryover in RNA samples can be tested by setting up a no-RT control reaction
- To set up a no-RT control reaction, simply replace the reverse transcriptase volume with nuclease-free water
- The same amount of total RNA should be used in both the RT and no-RT reactions to ensure similar carryover of cDNA synthesis components into a qPCR reaction

Recommendations for cDNA Archiving

cDNA can be stored at –20°C either undiluted or diluted in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA.

Related Products

Catalog #	Description
Reverse Transcription Reagents for Real-Time qPCR	
1708840	iScript Reverse Transcription Supermix 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/advancedcDNA** for more information.

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