BIO-RAD

iScript™ Advanced cDNA Synthesis Kit for RT-qPCR

170-8842 50 reactions iScript advanced cDNA synthesis kit for RT-qPCR
170-8843 250 reactions iScript advanced cDNA synthesis kit for RT-qPCR
For research purposes only.

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

The iScript advanced cDNA synthesis kit is an enhanced formulation that offers increased data throughput from a single 20 µl reverse transcription reaction for real-time qPCR. This 2-tube kit enables superior capacity as well as wide linear dynamic range for reverse transcription.

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

Kit Contents

Reagents	Description
5x iScript advanced reaction mix (red cap)	5x reaction mix with dNTPs, oligo(dT), random primers, buffer, $MgCl_2$, enhancers and stabilizers
iScript advanced reverse transcriptase (orange cap)	iScript MMLV-RT ($RNaseH^+$) and RNase inhibitor
Nuclease-free water	

Reaction Set Up for a Single cDNA Synthesis Reaction
For optimal results, reactions should be assembled on ice using appropriate reaction vessels.

Component	Volume per Reaction
5x iScript advanced reaction mix	4 µl
iScript advanced reverse transcriptase	1 µl
RNA template (100 fg to 7.5 µg)*	variable
Nuclease-free water	variable
Total volume	***20 µl***

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

Reaction Set Up for Multiple cDNA Synthesis Reactions
An example of master mix preparation for 10 reactions with 5 µl RNA input with 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.

Component	Volume per Reaction
5x iScript advanced reaction mix	48 µl
iScript advanced reverse transcriptase	12 µl
Nuclease-free water	120 µl
Total volume	***180 µl***

- Prepare the reverse transcription master mix as indicated in the table above. Mix thoroughly by pipetting up and down several times.
- Add 15 µl of the prepared master mix to 5 µl of total RNA input for each reverse transcription reaction.

- Adjust volume of water if RNA input volume differs from the above example. The final volume of master mix to be pipetted into each reaction should also be adjusted.

** If more reactions are required, scale up appropriately. The volume of components provided in 50 and 250 reaction kits does not take into account the preparation of excess master mix.

Reaction Protocol

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

Reverse transcription	30 min at 42°C
RT inactivation	5 min at 85°C

Recommendations for the use of no-RT Control

- Interference of gene expression analysis by genomic DNA carryover in RNA samples can be tested by setting up a no-RT control reaction.
- The reverse transcriptase volume in a no-RT control reaction should be substituted with water.
- The same amount of total RNA used in the +RT reaction should be used to ensure similar carryover of cDNA synthesis components in a qPCR reaction.

Recommendations for qPCR

- For RNA inputs 1.0 µg to 7.5 µg: cDNA generated with these inputs must be diluted at least 10-fold in 10mM Tris-HCL (pH 8.0), 0.1 mM EDTA or PCR-grade water prior to use in qPCR.
- Optimum cDNA dilution must be determined based on target gene abundance and qPCR chemistry.
- For input RNA less than 1 µg: 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.

Recommendations for cDNA Archiving

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

To learn more about Bio-Rad's complete solution for amplification, visit: **www.bio-rad.com/amplification**.

Bio-Rad Laboratories
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000

10022089 Rev A