

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 use 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

Guaranteed for 12 months at –20°C in a constant temperature freezer. See Table 1 for kit contents.

Table 1. 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 Setup and Protocol

For optimal results, reactions should be assembled on ice using sterile and nuclease-free tubes, tube strips, or plates.

- ❗ 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.
- ❗ Contamination by genomic DNA carryover in RNA samples can be tested by setting 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.

1. Prepare the cDNA synthesis reaction as indicated in Table 2. Mix thoroughly by pipetting up and down several times. If necessary, pulse centrifuge to collect the contents.

Table 2. Setup for cDNA synthesis reaction.

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	To 20
Total volume	20

- ❗ If multiple reactions are required, scale up appropriately. Prepare a master mix with excess reagents to accommodate loss during pipetting. Note that the kit component volumes provided in Table 2 do not take into account the preparation of excess master mix.

2. Incubate the complete reaction mix according to the guidelines in Table 3 using a thermal cycler with the heated lid on.

Table 3. cDNA synthesis reaction protocol.

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

- ❗ An alternate protocol (30 min at 42°C and 1 min at 95°C) can be used if needed. Both protocols yield equivalent cDNA synthesis results.

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.

Recommendations for qPCR

- For input RNA (1.0–7.5 µg): depending on target abundance, cDNA generated with this kit may have to 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

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/AdvancedcDNA for more information.

SYBR is a trademark of Life Technologies Corporation. Bio-Rad Laboratories, Inc. is licensed by Life Technologies Corporation to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.

The use of iTaq and SsoAdvanced Supermixes is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,804,375; 5,994,056; and 6,171,785. The purchase of these products includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, are conveyed expressly, by implication, or by estoppel. These products are for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.