

# iScript<sup>™</sup> Advanced cDNA Synthesis Kit for RT-qPCR

Catalog #	Description	
1725037	iScript Adva	
1725038	iScript Adva	

ipt Advanced cDNA Synthesis Kit for RT-qPCR, 25 x 20 µl reactions 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 gPCR 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.

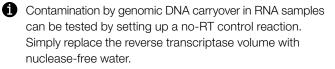
Reagent	Description
5x iScript Advanced Reaction Mix	5x reaction mix with dNTPs, oligo(dT), and random primers
iScript Advanced Reverse Transcriptase	RNase H+ Moloney murine leukemia virus (MMLV) reverse transcriptase and RNase inhibitor
Nuclease-free water	1.5 ml

# **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.



1 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-HCI (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-HCI (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 gPCR
- 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 <sup>™</sup> Universal SYBR <sup>®</sup> Green Supermix	
1725280	SsoAdvanced Universal Probes Supermix	
1725120	iTaq <sup>™</sup> Universal SYBR <sup>®</sup> Green Supermix	
1725130	iTaq Universal Probes Supermix	
1725160	SsoAdvanced PreAmp Supermix	

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