

# iScript™ cDNA Synthesis Kit

Catalog #	Description
1708890	<b>iScript cDNA Synthesis Kit</b> , 25 x 20 µl reactions
1708891	<b>iScript cDNA Synthesis Kit</b> , 100 x 20 µl reactions

**For research purposes only.**

## Introduction

iScript cDNA Synthesis Kit provides a sensitive and easy-to-use solution for two-step reverse transcription quantitative PCR (RT-qPCR). This kit includes three tubes, which contain all the reagents required for successful reverse transcription.

The iScript Reverse Transcriptase is RNase H<sup>+</sup>, which provides greater sensitivity than RNase H<sup>-</sup> enzymes in qPCR. iScript is a modified Moloney murine leukemia virus (MMLV) reverse transcriptase, optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. The enzyme is provided preblended with RNase inhibitor. The unique blend of oligo(dT) and random hexamer primers in the iScript Reaction Mix works exceptionally well with a wide variety of targets. This blend is optimized for the production of targets <1 kb in length. iScript cDNA Synthesis Kit produces excellent results in both real-time and standard RT-qPCR.

## Storage and Stability

Store at -20°C. Guaranteed for 12 months at -20°C in a constant temperature freezer. Nuclease-free water can be stored at room temperature.

**Note:** Kits whose six-digit lot number begins with a 2 are not compatible with kits whose six-digit lot number begins with a 1. Please make note of this distinction if you have multiple lots of this kit in storage.

## Kit Contents

Reagent	Volume for 25 Reactions	Volume for 100 Reactions
5x iScript Reaction Mix	100 µl	400 µl
iScript Reverse Transcriptase	25 µl	100 µl
Nuclease-free water	1.5 ml	1.5 ml

## Reaction Setup

**Note:** The 5x iScript Reaction Mix may generate some precipitation upon thawing; this does not affect the quality of the mixture. If you do experience precipitation, please mix thoroughly to resuspend and use as directed in the following table.

Component	Volume per Reaction, µl
5x iScript Reaction Mix	4
iScript Reverse Transcriptase	1
Nuclease-free water	Variable
RNA template (100 fg–1 µg total RNA)*	Variable
<b>Total volume</b>	<b>20</b>

\* When using larger amounts of input RNA (>1 µg), the reaction should be scaled up (for example, 40 µl reaction for 2 µg, or 100 µl reaction for 5 µg) to ensure optimum synthesis efficiency.

## 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
Optional step	Hold at 4°C

## Recommendation for Optimal Results Using the iScript cDNA Synthesis Kit

The maximum amount of the cDNA reaction that is recommended for downstream PCR is one-tenth of the reaction volume, typically 2 µl.

## Related Products

Catalog #	Description
<b>Reverse Transcription Reagents for Real-Time qPCR</b>	
1708840	iScript Reverse Transcription Supermix for RT-qPCR
1725037	iScript Advanced cDNA Synthesis Kit for RT-qPCR
1708896	iScript Select cDNA Synthesis Kit
1725034	iScript gDNA Clear cDNA Synthesis Kit
<b>Reagents for Real-Time qPCR</b>	
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

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