

iScript™ cDNA Synthesis Kit

25 x 20 µl reactions 170-8890

100 x 20 µl reactions 170-8891

For research purposes only

Store at -20°C (not frost-free)

iScript cDNA synthesis kit provides a sensitive and easy-to-use solution for two-step RT-PCR. This kit includes just three tubes — comprehensive of reagents required for successful reverse transcription.

The iScript reverse transcriptase is RNase H+, which provides greater sensitivity than RNase H- enzymes in quantitative PCR. iScript is a modified MMLV-derived 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 <1kb in length.

iScript cDNA synthesis kit produces excellent results in both real time and conventional RT-PCR.

Storage and Stability

Store the iScript™ cDNA synthesis kit at -20°C in a constant-temperature freezer. When stored under these conditions, the kit components are stable for a minimum of one year after ship date. Nuclease-free water can be stored at room temperature.

Important Note

We have recently made improvements to our manufacturing procedure, in order to ensure that you receive the highest quality product we can deliver. 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

Reagents	Volume
25 Reaction Kit	
5x iScript reaction mix	100 µl
Nuclease-free water	1.5 ml
iScript reverse transcriptase	25 µl

100 Reaction Kit	
5x iScript reaction mix	400 µl
Nuclease-free water	1.5 ml

Reaction Setup

Please 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 below.

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

Reaction Protocol

- Incubate complete reaction mix:
 - 5 minutes at 25°C
 - 30 minutes at 42°C
 - 5 minutes at 85°C
 - Hold at 4°C (optional)

Reagents and Materials Not Supplied

- Reagents for PCR or real-time PCR
 - Such as:
 - iTaq™ DNA polymerase, 170-8870
 - iQ™ supermix, 170-8860, or
 - iQ™ SYBR® Green supermix, 170-8880
- Pipet tips, aerosol barrier tips
 - Such as:
 - Xcluda™ style B, 211-2006
- Nuclease-free tubes
 - 0.2 ml thin-wall tubes TWI-0201
 - 0.2 ml thin-wall tubes HSP-9601 (Low-Profile)
 - HSS-9601 (Full Height)
- RNA purification kit
 - Such as:
 - Aurum™ total RNA mini kit, 732-6820 or
 - Aurum total RNA kit, 2 x 96 well, 732-6800

Recommendations 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.

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

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

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