

AMPLIFICATION

iScript™ Advanced cDNA Synthesis Kit for RT-qPCR

Increased qPCR data throughput
Advanced formulation for superior dynamic range (100 fg–7.5 µg)

Obtain More Data from a Single Reaction

The iScript advanced cDNA synthesis kit for RT-qPCR is an enhanced formulation that delivers increased data throughput from a single 20 µl reverse transcription (RT) reaction for real-time qPCR. This two-tube kit enables superior RT capacity of input RNA up to 7.5 µg.

Increase qPCR data throughput and cost effectiveness from a single reaction — cDNA synthesized from higher input RNA amounts can be used for analyzing large numbers of target genes

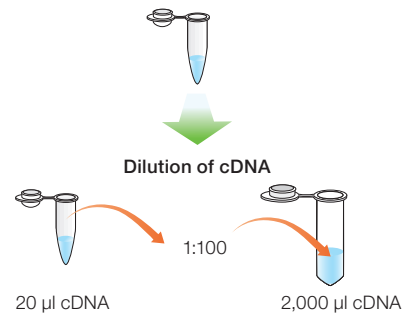
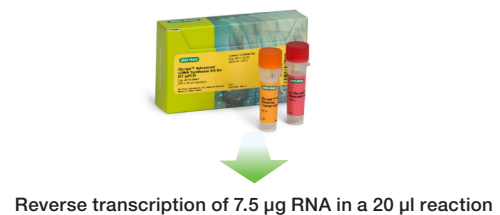
Detect low-level target genes — uncompromised sensitivity even with less input RNA for when RNA sample is limited

Reduce inter-assay variability and improve reproducibility — eliminate the need for multiple cDNA synthesis reactions

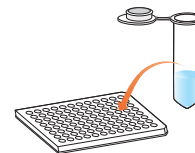
Allow true representation of target mRNA levels — potent blend of RNase inhibitor prevents RNA degradation during reaction setup and RT

Increase primer design flexibility and prevent 5' and 3' bias — optimum blend of oligo(dT) and random primers for superior RNA coverage

Set up reactions with ease and obtain cDNA in less time — 2-tube kit generates cDNA in 35 min

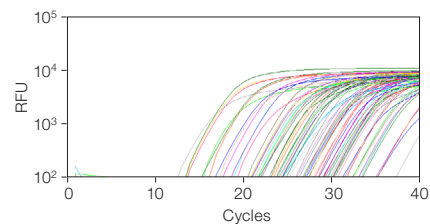


Real-time qPCR for 96 targets on a PCR array

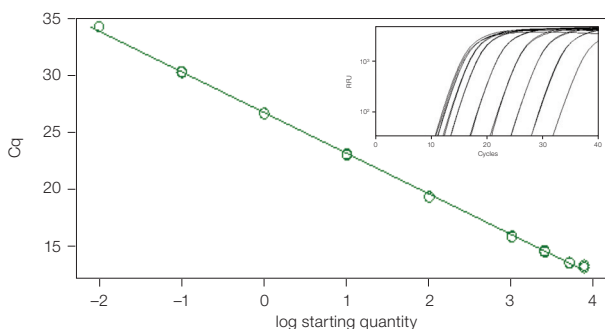


Add 2 µl cDNA to each well of a 96-well plate (192 µl cDNA/plate)

Up to 1,000 RT-qPCR data points from a single 20 µl RT reaction (96-well plate shown)

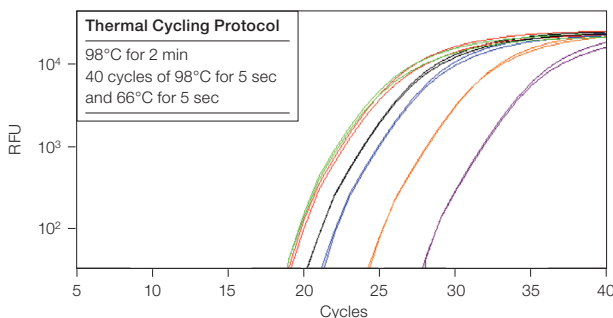


Extended Dynamic Range and Higher Capacity



Total RNA (7.5 µg–1 µg) from HeLa cells was reverse transcribed using the iScript advanced cDNA synthesis kit for RT-qPCR in a 20 µl reaction. A tenfold dilution of generated cDNA was used as template to amplify α -tubulin in a 10 µl qPCR reaction with iQ™ SYBR® Green supermix on a CFX384™ real-time PCR detection system. Efficiency = 90.7%, R² = 0.999, slope = -3.57. Cq, quantification cycle; RFU, relative fluorescence units.

Efficient Amplification of High GC Targets

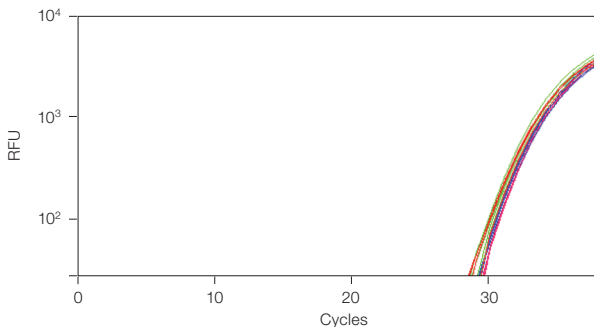


Six different input RNA amounts (7.5, 5, 2, 1, 0.1, and 0.01 µg) from HeLa cells were reverse transcribed using the iScript advanced cDNA synthesis kit for RT-qPCR in a 20 µl reaction. The generated cDNA was diluted 20-fold for qPCR. A 110 bp CBP amplicon (70% GC) was amplified in a 10 µl qPCR reaction with SsoFast™ EvaGreen® supermix on a CFX96™ real-time PCR detection system. CBP efficiency = 99%, R² = 0.998, slope = -3.33. RFU, relative fluorescence units.

Ordering Information

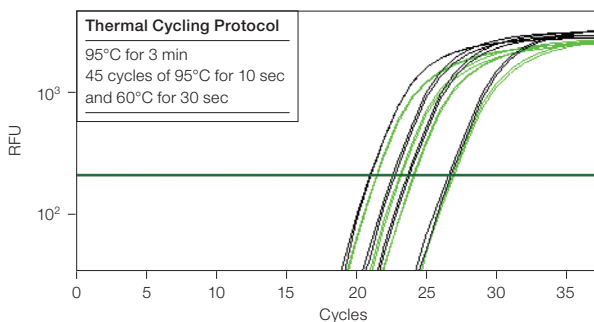
Catalog #	Description
170-8842	iScript Advanced cDNA Synthesis Kit for RT-qPCR , 50 x 20 µl reactions, includes 200 µl 5x iScript advanced reaction mix, 50 µl iScript advanced reverse transcriptase
170-8843	iScript Advanced cDNA Synthesis Kit for RT-qPCR , 250 x 20 µl reactions, includes 1,000 µl 5x iScript advanced reaction mix, 250 µl iScript advanced reverse transcriptase

Accurate mRNA Representation



Total RNA (7.5, 5, 2.5, and 1 µg) from human spleen was reverse transcribed using the iScript advanced cDNA synthesis kit for RT-qPCR. The generated cDNA samples were diluted to the equivalent of 50 ng/µl and used as template to amplify a low-expressed gene (IL-2) in a 10 µl qPCR reaction with SsoFast™ probes supermix on a CFX384 real-time PCR detection system (FAM probe). There were no significant differences (<0.5 Cq) between diluted samples, which demonstrates accurate representation of mRNA transcripts for high and low input RNA. RFU, relative fluorescence units.

Superior 5' and 3' Coverage



Total RNA (7.5, 2, 1, and 0.1 µg) from HeLa cells was reverse transcribed using the iScript advanced cDNA synthesis kit for RT-qPCR in a 20 µl reaction. One-tenth of the generated cDNA was used as template to amplify the APC gene in a 10 µl qPCR reaction with iQ™ SYBR® Green supermix on a CFX384 system. Primer pairs were designed at the 5' end () and 3' end () for 91 and 69 bp amplicons. There was no significant difference between the two primer pairs, which demonstrates superior coverage of the 5' and 3' regions of target mRNA. RFU, relative fluorescence units.

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

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applera's U.S. Patent Number 6,814,934 B1 for use in research, human in vitro diagnostics, and all other fields except veterinary diagnostics.

Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

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