AMPLIFICATION

iScript™ Reverse Transcription Supermix for RT-qPCR

- Reduces effect of pipetting variability and errors during setup
- Detects low-level target genes with real-time qPCR

Fast, Sensitive, and Reproducible — Just Add RNA

The iScript reverse transcription supermix for RT-qPCR is a simple, fast, and sensitive first-strand cDNA synthesis kit for gene expression analysis using real-time qPCR. In one tube, the preblended 5x supermix contains all the necessary components except RNA template for reverse transcription.

- **Increase reproducibility of reverse transcription and reduce errors during setup** — 1-tube format for simple and fast setup and reduced pipetting variability
- **Detect low-level target genes and conserve RNA during gene expression analysis** — broad linear dynamic range of total input RNA (1 µg–1 pg) with a highly efficient RNase H+ MMLV reverse transcriptase
- **Use with dilute RNA samples** — 5x concentration of supermix allows for greater volume of RNA sample in a 20 µl cDNA synthesis reaction
- **Eliminate freeze/thaw cycles** — liquid format at –20°C shortens overall processing time and maximizes stability
- **Validate purity of input RNA and gene expression results** — optional no-RT controls for detecting genomic DNA contamination
- **Obtain accurate results** — potent blend of RNase inhibitor prevents RNA degradation during reaction setup and reverse transcription
- **Increase primer design flexibility and prevent 5' and 3' bias** — optimum blend of oligo(dT) and random primers in the 5x supermix for complete RNA coverage
- **Complete cDNA synthesis and qPCR the same day** — fast 40 min cDNA synthesis protocol

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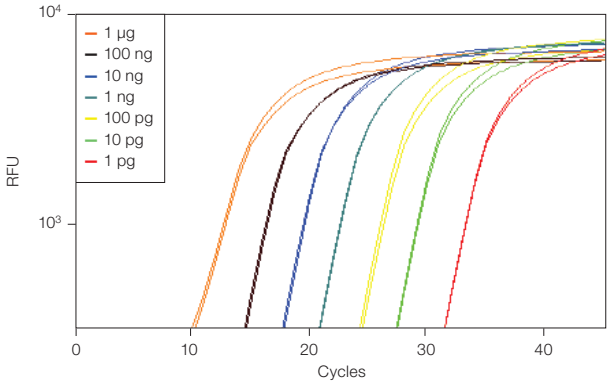
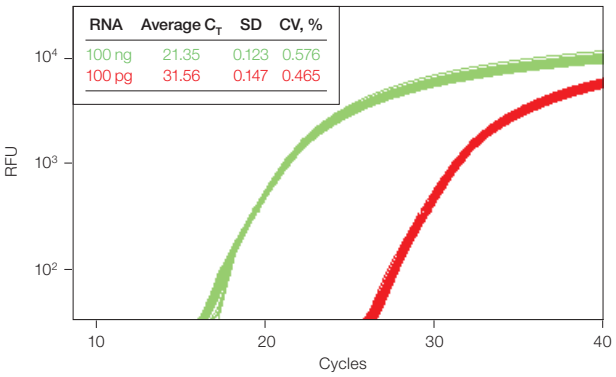
Broad Linear Dynamic Range



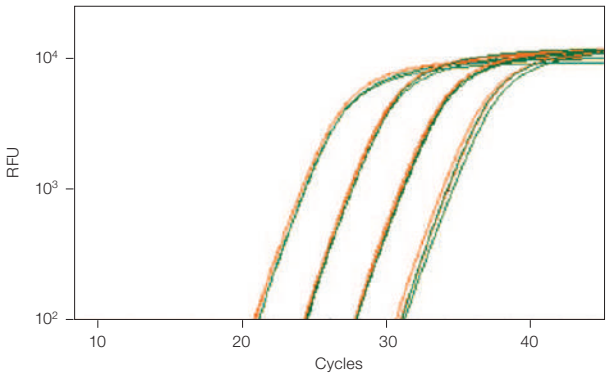
The iScript reverse transcription supermix for RT-qPCR efficiently reverse transcribes RNA over a broad linear dynamic range for reliable gene expression analysis data. Different amounts of HeLa cell RNA (amounts shown in inset) were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify β-actin gene (~90 bp) in 20 µl qPCR reactions with iQ™ SYBR® Green supermix. Standard curve r^2 = 0.999, efficiency = 99.7%, slope = –3.33. RFU, relative fluorescence units.

Data Reproducibility



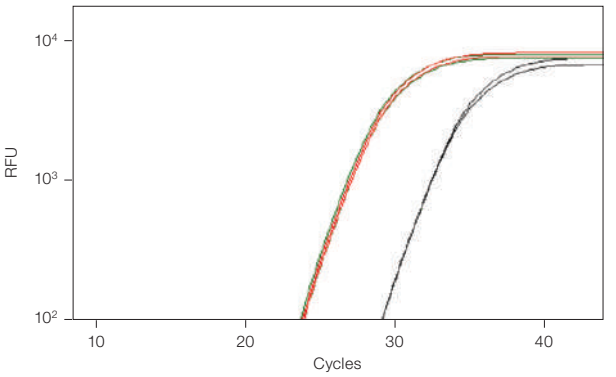
Excellent data reproducibility. *PGK-1* mRNA (~160 bp), a gene that encodes a glycolytic enzyme, was quantified using iScript reverse transcription supermix for RT-qPCR both with 100 ng (■) and 100 pg (■) of input RNA. For each input RNA, 48 individual RT reactions were performed and one-tenth of resulting cDNA was used in the qPCR reaction with SsoFast™ probes supermix. The gene expression analysis data show excellent reproducibility both with high and low levels of input target mRNA. The ~10 threshold cycle (C_T) difference for the 1,000-fold dilution of RNA (100 ng–100 pg) demonstrates good reverse transcription efficiencies across different input RNAs. RFU, relative fluorescence units.

Unbiased Representation of 5' and 3' Regions



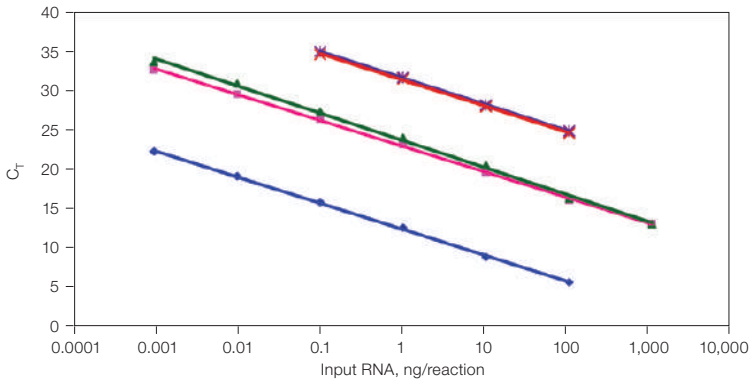
Unbiased representation of 5' and 3' regions of target genes. Reverse transcription of 100, 10, 1, and 0.1 ng input RNA was performed with iScript reverse transcription supermix for RT-qPCR. Primer pairs were designed at 5' (■, ~60 bp) and 3' (■, ~70 bp) ends of the *MAP* gene and qPCR was performed with one-tenth of input cDNA using iQ™ SYBR® Green supermix. There were no significant differences (<0.5 C_T difference) between the two primer pairs, which demonstrates unbiased representation of 5' and 3' regions. RFU, relative fluorescence units.

Protection of RNA Integrity



Potent RNaseA inhibition. iScript reagents for RT-qPCR include an optimum blend of RNaseA inhibitor for protecting RNA integrity. Reverse transcription was performed using 0.1 pg of input RNA with iScript reagent alone (■), spiked with RNaseA (■), or spiked with RNaseA without the RNaseA inhibitor included in the reaction (■). 18S rRNA (~70 bp) was amplified using iQ™ SYBR® Green supermix. A significant C_T delay was observed when the reaction included RNaseA but no RNaseA inhibitor, which demonstrates potent RNaseA inhibition. RFU, relative fluorescence units.

Maximum Efficiency and Sensitivity with SYBR® Green



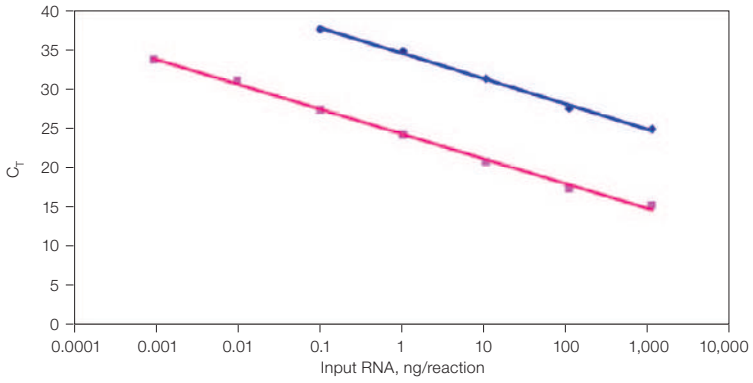
Gene	Slope	Efficiency	R^2
18S rRNA	–3.33	99.8%	0.9995
β-actin	–3.28	101.7%	0.9994
GAPDH	–3.47	94.3%	0.9976
MAP 5' amplicon	–3.33	99.5%	0.9994
MAP 3' amplicon	–3.36	98.3%	0.9998

Thermal Cycling Protocol

95°C for 5 min
45 cycles of 95°C for 15 sec and 60°C for 30 sec

SYBR® Green compatibility with iScript reverse transcription supermix for RT-qPCR. Different amounts of HeLa cell RNA were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify 18S rRNA (~70 bp), β-actin (~90 bp), *GAPDH* (~200 bp), and *MAP* (5' amplicon: ~60 bp; 3' amplicon: ~70 bp) genes in 20 µl qPCR reactions with iQ™ SYBR® Green supermix. C_T, threshold cycle.

Maximum Efficiency and Sensitivity with EvaGreen



Gene	Slope	Efficiency	R^2
CBP (70% GC)	–3.26	102.7%	0.9964
β-actin	–3.19	106.0%	0.9969

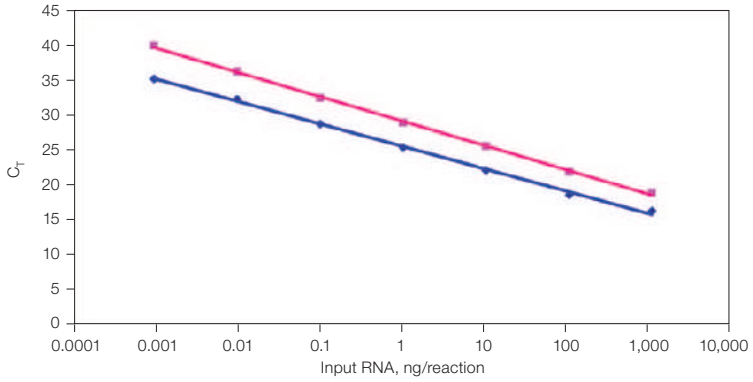
Thermal Cycling Protocol

***CBP* gene**
95°C for 30 sec
40 cycles of 95°C for 5 sec and 66°C for 10 sec

β-actin gene
95°C for 30 sec
40 cycles of 95°C for 5 sec and 60°C for 5 sec

EvaGreen and high-GC amplicon compatibility with iScript reverse transcription supermix for RT-qPCR. Different amounts of HeLa cell RNA were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify *CBP* (~100 bp) and β-actin (~90 bp) genes in 20 µl qPCR reactions with SsoFast™ EvaGreen® supermix. C_T, threshold cycle.

Maximum Efficiency and Sensitivity with SsoFast Probes Supermix



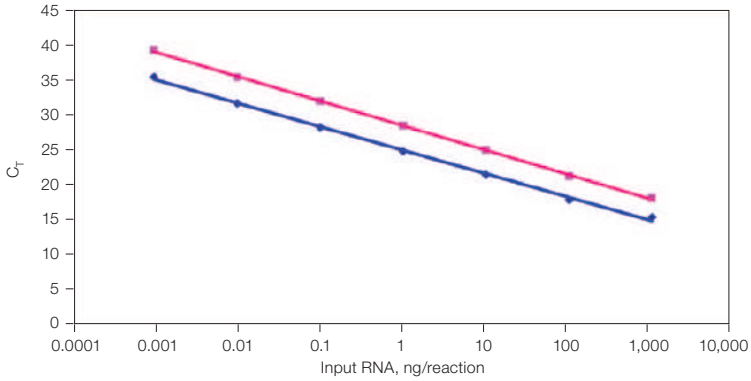
Gene	Slope	Efficiency	R^2
β-actin	–3.23	104.1%	0.9980
α-tubulin	–3.50	92.9%	0.9992

Thermal Cycling Protocol

95°C for 10 sec
40 cycles of 95°C for 3 sec and 60°C for 20 sec

Probe-based qPCR with SsoFast probes supermix. Different amounts of HeLa cell RNA were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify β-actin (~90 bp) and α-tubulin (~120 bp) genes in 20 µl qPCR reactions. C_T, threshold cycle.

Maximum Efficiency and Sensitivity with iQ™ Supermix



Gene	Slope	Efficiency	R^2
β-actin	–3.34	99.4%	0.9980
α-tubulin	–3.48	93.7%	0.9992

Thermal Cycling Protocol

95°C for 5 min
44 cycles of 95°C for 15 sec and 60°C for 30 sec

Probe-based qPCR with iQ supermix. Different amounts of HeLa cell RNA were reverse transcribed and one-tenth of resulting cDNA was used as template to amplify β-actin (~90 bp) and α-tubulin (~120 bp) genes in 20 µl qPCR reactions. C_T, threshold cycle.

Ordering Information

Catalog #	Description
170-8840	**iScript Reverse Transcription Supermix for RT-qPCR**, 25 x 20 µl reactions, 100 µl of 5x supermix contains reverse transcriptase, RNase inhibitor, dNTPs, primers, $MgCl_2$, stabilizers; 10 reactions of no-RT control supermix
170-8841	**iScript Reverse Transcription Supermix for RT-qPCR**, 100 x 20 µl reactions, 4 x 100 µl of 5x supermix contains reverse transcriptase, RNase inhibitor, dNTPs, primers, $MgCl_2$, stabilizers; 10 reactions of no-RT control supermix
Related qPCR Supermixes	
170-8860	**iQ Supermix**, 100 x 50 µl reactions, 2x mix contains 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 50 U/ml iTaq™ DNA polymerase, 6 mM $MgCl_2$, stabilizers
170-8880	**iQ™ SYBR® Green Supermix**, 100 x 50 µl reactions, 2x mix contains 100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 mM each dNTP (dATP, dCTP, dGTP, dTTP), 50 U/ml iTaq DNA polymerase, 6 mM $MgCl_2$, SYBR® Green I, 20 nM fluorescein, stabilizers
172-5200	**SsoFast EvaGreen Supermix**, 200 x 20 µl reactions, 2x mix contains dNTPs, Sso7d fusion polymerase, $MgCl_2$, EvaGreen dye, stabilizers
172-5230	**SsoFast Probes Supermix**, 200 x 20 µl reactions, 2x mix contains dNTPs, Sso7d fusion polymerase, $MgCl_2$, stabilizers

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