ZYMO RESEARCH

ZymoScript™ One-Step RT-qPCR Kit

Simple and sensitive qPCR detection of RNA targets

Cat. No. R3014 (100 Reactions)

Storage: -20 °C

Product Information

Features:

- Versatile and fast RT-qPCR compatible with both SYBR Green and TaqMan-based assays
- Efficient cDNA synthesis coupled with robust amplification
- Reduced non-specific product formation
- Ideal for pathogen detection and gene expression analysis
- Tracking dye to monitor pipetting errors

Description:

The **ZymoScript™ One-Step RT-qPCR Kit** contains all the necessary components for fast reverse transcription and robust qPCR amplification in a single reaction. Simply add RNA template and primers to achieve streamlined RT-qPCR in a single tube.

ZymoScript™ One-Step RT-qPCR Kit is compatible with both SYBR Green and TaqMan probe-based assays for ultimate flexibility. The provided Fluor Dye solution can be added to the RT-qPCR reaction for SYBR Green-based detection. Alternatively, TaqMan probes can be added for multiplex RT-qPCR analysis. cDNA synthesis and robust target amplification can be achieved in 1.5 hour. The provided enzyme mix contains optimized elements for maximum specificity during each reaction step.

The resulting RT-qPCR product contains an "A" overhang to allow for TA-cloning.

Product Contents:

	Catalog Number	R3014 (100 reactions)	Storage Temp.
Reaction Buffer (4X)	R3014-1-500	1 x 500 µl	-20 °C
Enzyme Mix (20X)	R3014-2-100	1 x 100 µl	-20 °C
Fluor Dye (20X)	R3014-3-100	1 x 100 µl	-20 °C
$MgCl_2$ (25 mM)	R3014-4-500	1 x 500 µl	-20 °C
DNase/RNase-Free Water	W1001-1	1 x 1 ml	RT

Note: Fluor Dye must be protected from light.

Storage:

Store at -20 °C. Minimize exposure to light.

Suggested Reaction Setup (20 µl):

Reagent	Volume	Final conc.
Reaction Buffer (4X)	5.0 µl	1X
Enzyme Mix (20X)	1.0 µl	1X
Forward Primer (10 µM)	0.8 µl	0.4 µM
Reverse Primer (10 µM)	0.8 µl	0.4 µM
Fluor Dye (20X)*	1.0 µl	1X
*For SYBR Green assays only		
Template RNA	variable	0.1 pg to 5 µg total
DNase/RNase-Free Water	to 20 µl	–
Total volume	20 µl	

Notes:
- Only add Fluor Dye if performing SYBR Green-based fluorescent detection.
- $MgCl_2$ is present in the reaction buffer. Its concentration in the final RT-qPCR reaction is 1.6 mM, but can be increased up to 4 mM using the provided 25 mM $MgCl_2$.

Suggested Conditions For RT-qPCR:

1) Primer annealing & cDNA synthesis[1]	55 °C	10 min.
2) Initial denaturation	95 °C	10 min.
3) Denaturation	95 °C	20 sec.
4) Annealing/Extension[2]	60 °C	60 sec. for ≤ 1kb
5) Plate Read		
30-45 cycles		
6) Optional: Melt Curve	60-95 °C	
*For SYBR Green assays only		

[1] For the cDNA synthesis of long transcripts, extend this step up to 45 min.

[2] Optimal annealing temperature may vary based on primer design. It is recommended to use an annealing temperature at least 2°C below the lowest melting temperature of the primers. Add 60 seconds to the extension time for each additional kb over 1 kb.

- If cloning after RT-qPCR is desired, include a final extension step of 72°C for 5 minutes.

Related Products:

Product	Cat. Number	Description
***Quick*-RNA™ Kits**	R1050	RNA isolation from wide range of cell and tissue samples
Direct-zol™ RNA Kits	R2050	RNA extraction from samples in TRI Reagent® or similar
DNase I Set	E1010	DNase I enzyme and digestion buffer
ZymoScript™ RT PreMix Kit	R3012	RT kit, ready to use PreMix formulation. Use for 2-step RT-qPCR

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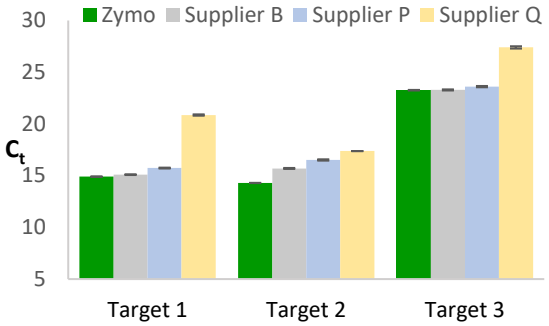
Additional Information

ZymoScript™ One-Step RT-qPCR Analysis:

The ZymoScript™ One-Step RT-qPCR Kit enables highly sensitive target detection compared to other suppliers



RNA was isolated from HeLa cells. Values are represented as mean (+/- SE) cycle threshold (C_t) of 3 RT-qPCR technical replicates. Targets 1, 2 and 3 are GAPDH, ACTB, and TBP, respectively.

FAQ:

1. What is the minimum/maximum amount of RNA template I should use?

The amount of total RNA required may vary depending on the expression level of the target transcripts. In general, we recommend using 0.1 pg - 5 µg of input RNA.

2. Will the blue tracking dye interfere with qPCR fluorophore channels and affect the signal of probe/fluorescent dyes?

The dye used in the ZymoScript™ One-Step RT-qPCR Kit has been thoroughly tested in downstream analysis. At the provided concentration, it will not affect fluorescent signal readings during qPCR.

3. How is the performance of the ZymoScript™ One-Step RT-qPCR Kit with low-quality RNA samples?

Lower signal can be expected with degraded and impure RNA samples. We recommend using *Quick*-RNA™ or Direct-zol™ Kits (see **Related Products**) for high quality RNA extraction.

4. RT-qPCR signal is not detected, or it is detected at higher Ct than expected. What should I do?

A possible reason for missing or delayed amplification is inefficient primer binding to the template. Make sure to use an annealing temperature (step 4 in the protocol provided) at least 2°C below the lowest melting temperature of the primers to ensure efficient binding to the target sequence.

5. Can I assemble the RT-PCR reaction at room temperature?

Yes, the reaction can be assembled at room temperature. The enzymes are only activated at higher temperatures, ensuring maximum specificity.

6. Can I store the ZymoScript™ One-Step RT-qPCR Kit at Room Temperature or 4 °C?

We do not recommend storing the kit or kit components at room temperature or at 4 °C for prolonged periods of time.

7. How can I ensure that there is no genomic DNA contamination in my RT reaction?

Samples can be treated with DNase I to eliminate DNA contamination. DNase I is included in the *Quick*-RNA™ or Direct-zol™ Kits recommended for RNA extraction. Alternatively, the DNase I set (see **Related Products**) can be purchased separately.

8. Do I need to add RNase inhibitors?

No, RNase inhibitors are already included in the enzyme mix to prevent unwanted RNA degradation during reverse transcription.

9. Should I use SYBR Green or a TaqMan probe-based assay?

For single target detection, SYBR Green-based assays offer a more affordable solution than TaqMan probe-based assays. For SYBR Green-based assays, add 1 µl of Fluor Dye solution (20X) to each 20 µl RT-qPCR reaction. For TaqMan probe-based assays we recommend adding TaqMan probes at a final concentration of 0.2 µM. Do not add Fluor Dye solution to the RT-qPCR reaction when performing TaqMan probe-based assays.

10. What fluorophores are recommended for TaqMan probe-based assays?

In general, any commonly used fluorophore (including HEX, VIC, Cy3, Texas Red, Cal Red 610, Quasar 670, Cy5, Cy5.5, and Quasar 705) is compatible with this kit with the exception of FAM fluorophore, which is unstable in our buffer system. Instead, we recommend using Alexa Fluor™ 488, which has overlapping excitation and emission spectra with FAM, but greater stability.

11. Which channel should be activated when using the Fluor Dye solution?

The Fluor Dye has the same excitation and emission properties as SYBR Green. Activate the SYBR/FAM channel for detection when using the Fluor Dye.

12. Does ZymoScript™ One-Step RT-qPCR Kit contain a passive reference dye?

No, a passive reference dye is not included. This allows to maximize the number of targets for multiplex assays.

13. What is the amplicon length that can be achieved using the ZymoScript™ One-Step RT-qPCR Kit?

This depends on different factors, including RNA integrity and primer efficiencies. Generally, this product performs the best with amplicons with a length between 50 bp and 1.5 Kb.

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