ZYMO RESEARCH

ZymoScript™ RT PreMix Kit

Simple and fast cDNA synthesis from RNA

Cat. No. R3012 (100 Reactions)

Storage: -20 °C

Product Information

Features:

- Simple ready-to-use 2X formulation
- Efficient and quick cDNA synthesis
- Reverse Transcription of up to 5 µg of RNA
- Tracking dye to monitor pipetting errors

Description:

The **ZymoScript™ RT PreMix Kit** is an optimized 2X formulation that includes all the necessary components needed to perform robust reverse transcription. This includes random hexamer and oligo dT primers, dNTPs, RNase inhibitors, reverse transcriptase, and an optimized buffer for efficient cDNA synthesis.

After the RNA sample is added to ZymoScript™ RT PreMix, the reaction is incubated at 42-55 °C to initiate the reverse transcription step. High yield, full-length cDNA synthesis occurs in less than 15 minutes. After the RT enzyme is inactivated, the reaction mixture is ready for downstream analysis such as qPCR. ZymoScript™ RT PreMix Kit is compatible with SYBR Green or TaqMan probe-based assays. We recommend using Zymo*Taq* qPCR PreMix (Cat. No E2054) for qPCR analysis of the diluted or undiluted cDNA product.

To make reaction set up even easier, the 2X ZymoScript™ RT PreMix formulation includes a blue dye for easy tracking of pipetting. This dye does not interfere with downstream analyses, including fluorescent detection methods.

Product Contents:

	Catalog Number	R3012 (100 Rxns.)	Storage Temp.
ZymoScript™ RT PreMix (2X)	R3012-1-1	1 x 1 ml	-20 °C
DNase/RNase-Free Water	W1001-1	1 x 1 ml	RT

Storage:

Store at -20 °C. Avoid repeated freeze/thawing of reagents.

Suggested Reaction Setup (20 µl):

Reagent	Volume	Final conc.
2X ZymoScript™ RT PreMix	10 µl	1X
Experimental RNA Input	x µl	Up to 5 µg
DNase/RNase-Free Water	to 20 µl	-
Total volume	20 µl	

Note: Reactions can be assembled at room temperature. After adding reaction components, mix and centrifuge briefly. Place in thermal cycler using the following parameters:

Suggested Conditions for Reverse Transcription:

1) Incubation	25 °C	2 min
2) Extension[1]	42 °C	10 min
3) RT Inactivation	95 °C	1 min

[1] If cDNA synthesis of high GC content regions is desired, increase the temperature of step 2 up to 55°C. For the cDNA synthesis of long transcripts, it is recommended to extend the duration of step 2 up to 1 hour.

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Additional Information

FAQ:

1. What is the minimum/maximum amount of RNA template I can 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 ZymoScript™ RT PreMix has been thoroughly tested in downstream analyses. At the provided concentration, it will not affect fluorescent signal readings of any qPCR fluorescent channel.

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

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

4. Can I use gene specific primers to increase the cDNA synthesis of a target gene?

Yes, gene specific primers can be used to increase specific target yield. However, we recommend using ZymoScript™ One Step RT-qPCR Kit (see **Related Products**) for enhanced specificity.

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

Yes, the reaction can be assembled at room temperature.

6. Can I store the ZymoScript™ RT PreMix Kit at Room Temperature or 4°C?

We do not recommend storing the kit at room temperature for prolonged periods of time. The ZymoScript™ RT PreMix can be stored at 4°C for up to 1 week.

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™ and Direct-zol™ Kits recommended for RNA extraction (see **Related Products**). Alternatively, the DNase I set 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. Can I use the ZymoScript™ RT PreMix Kit to generate long cDNA for gene/cDNA cloning?

No. The ZymoScript™ RT PreMix (2X) already contains a mixture of oligo dT and random hexamer primers which is not ideal for the synthesis of long cDNA molecules. For this specific application we recommend using the ZymoScript™ One-Step RT-qPCR Kit (R3014) with gene-specific primers.

10. ZymoScript™ RT PreMix does not freeze at -20°C. Is this normal?

Yes, this is normal. ZymoScript™ RT PreMix will remain liquid at -20°C.

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 TRIzol® or similar reagents
DNase I Set	E1010	DNase I enzyme and digestion buffer
ZymoTaq qPCR PreMix	E2054	Recommended for qPCR following reverse transcription.
ZymoScript™ One-Step RT-qPCR Kit	R3014	One-Step RT-qPCR kit. Alternative to RT kit.