OneScript® RT Mix for qPCR w/gDNAOut

Reference: OZYA012-10 / OZYA012-100

SIZE: 10 rxn / 100 rxn (20 µL/rxn)
STORAGE: -20°C upon arrival
SHELF-LIFE: one year from the date of reception when stored properly

Product Description:

OneScript® RT Mix for qPCR with gDNAOut is an efficient and fast first-strand cDNA synthesis master mix, suitable for two-step RT-qPCR detection. The 5X **OneScript® RT Mix** in this product contains all the relevant reagents required for the reverse transcription reaction. It is only needed to add RNA template and DNase/RNase-free water to perform the reverse transcription reaction. The protocol is simple. The **OneScript® Reverse Transcriptase** in the master mix is a new-generation reverse transcriptase developed based on M-MuLV Reverse Transcriptase, which has reduced RNase H activity and improved thermal stability. The reaction temperature can be settled to up to 55°C, which greatly improves reverse transcription reaction efficiency and tolerance of complex RNA transcripts (high secondary structures or high GC contents), with higher specificity and higher yield.

The gDNAOut Mix in this product can completely remove residual genomic DNA in RNA samples and makes the quantitative results more accurate. It is heat sensitive and can be rapidly and irreversibly inactivated by heating at high temperature. So only sample addition is required, and the reaction can be carried out in the same tube to remove genomic DNA contamination and to reverse transcribe the RNA. This cDNA product is specially optimized for qPCR. The primer mix with optimized balance of Random Primers/Oligo $d(T)_{20}$VN Primers enables that the cDNA synthesis starts at any region of the RNA transcript and with the same reverse transcription efficiency, ensuring high authenticity and reproducibility of qPCR results.

The reverse transcription product is compatible with SYBR® Green and probe method qPCR. According to the purpose of the experiment, the qPCR reagents can be selected to have high-performance gene expression analysis.

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List of Components:

Component	OZYA012-10	OZYA012-100
5X OneScript® RT Mix*	40 µL	400 µL
20X gDNAOut Mix	10 µL	100 µL
DNase/RNase free water	1.25 mL	2 x 1.25 mL

**5X OneScript® RT Mix contains OneScript® Reverse Transcriptase, RNase Inhibitor, dNTPs, Random Primers/Oligo(dT)$_{20}$VN Primer Mix etc.*

Precautions:

1. When using 5X OneScript® RT Mix, please fully thaw it and mix it well before use. When multiple 5X OneScript® RT Mix reactions need to be prepared at the same time, the required working solution should be prepared in advance, and then aliquoted into each reaction tube to reduce reagent loss.
2. Random Primer and Oligo dT Primer are included in the 5X OneScript® RT Mix, thus gene-specific primers cannot be used.
3. Before aliquoting, please centrifuge and collect the reagents to the bottom of the tube. Immediately put it back into the refrigerator at -20°C after use.
4. Non-polluting pipette tips and microtubes must be used for the preparation and dispensing of the reaction solution to avoid contamination. Replace pipette tips each time when pipetting new reagent to avoid cross-contamination.

Standard protocol:

1. **Prepare the following reaction mix on ice**:
   Add the components to the DNase/RNase-free PCR tube on ice according to the following recommendations, mix well and centrifuge briefly:

Reagent	20 µL
5X OneScript® RT Mix	4 µL
20X gDNAOut Mix	1 µL
Total RNA	1 pg – 1 µg
DNase/RNase free water	to 20 µL

2. **Set the RT reaction**:

Temperature	Time
37°C	2 min.
55°C	15 min.
85°C	5 min.
4°C	Hold

Note: The cDNA product (RT reaction solution) can be used immediately for subsequent qPCR reactions, or stored at -20°C and used within half a year; for long-term storage, it is recommended to aliquot the cDNA product and to store the aliquots at -80°C. Repeated freezing and thawing of cDNA should be avoided.

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RT-qPCR considerations and optimizations:

RNA samples:

Complete and high-quality RNA is essential for obtaining high quality cDNA. Ensure RNA is not degraded or contaminated before the experiment. If RNA molecules contain complex secondary structures or high GC content, heat the RNA samples at 65 ℃ for 5 minutes and put it immediately on ice before the reverse transcription.

5X OneScript® RT Mix:

The RT mix already contains Oligo(dT)$_{20}$VN and random primers. It is suitable for eukaryotic mRNA with Poly(A) tail and for prokaryotic RNA, eukaryotic rRNA or tRNA without Poly(A) tail templates, but it is not suitable for small RNA templates such as miRNA.

The reverse transcription product (cDNA) obtained by using this product is only suitable for qPCR reaction and is not suitable for long fragment PCR amplification in downstream experiments such as cloning.

cDNA:

The cDNA products obtained after the reverse transcription reaction can be directly used for the qPCR reactions. It is recommended that the volume of undiluted cDNA product should not exceed 1/10 of the qPCR reaction volume or use Nuclease-free H_2O to dilute cDNA before adding it to the qPCR reaction.

qPCR mix selection guide:

We recommend use the ***ONEGreen®*** Fast qPCR Premix (OZYA008) for SYBR® Green qPCR detections. Any commercial qPCR premix for probe-based detection can be used after the OneScript® RT Mix for qPCR with gDNAOut to perform two-step qPCR.

FOR ORDERING:

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Document version:

OZYA012-1121

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