

GDSlyo One-step Probe RT-qPCR Kit

Cat. No./Spec.

Cat. No.	V5013-A	V5013-B	V5013-C
20- μ l reaction Nos.	200 rxns	1,000 rxns	5,000 rxns

Components

Component	V5013-A	V5013-B	V5013-C
5X GDSlyo One-step Buffer ^[1]	800 μ l	4 ml	20 ml
GDSlyo One-step Enzyme Mix ^[2]	200 μ l	1 ml	5 ml
3X Excipient ^[3]	1.2 ml	6 ml	30 ml
50X ROX Reference Dye 1 ^[4]	80 μ l	400 μ l	1 ml \times 2
50X ROX Reference Dye 2 ^[4]	80 μ l	400 μ l	1 ml \times 2
RNase-free ddH ₂ O	1 ml \times 3	15 ml	75 ml

[1] Contains dNTP/dUTP mix, Mg²⁺.

[2] Contains Reverse Transcriptase, RNase inhibitor, Heat-labile UDG and Taq DNA Polymerase.

[3] Lyophilized excipients, protect the activity of enzymes during the lyophilization process, maintain the morphology and long-term storage stability of lyophilized products.

[4] Used to correct the fluorescence signal error between holes. 50X ROX Reference Dye 1 is used for ABI 7900HT/ 7300 real-time PCR System and StepOne Plus; ABI 7500, 7500 Fast real-time PCR System, Stratagene Mx3000P using 50X ROX Reference Dye 2; Roche, bio-rad Real Time PCR Systems do not have to use ROX.

Storage

This reagent should be kept at -20°C.

Description

The GDSlyo One-step Probe RT-qPCR Kit is a glycerol-free one-step RT-qPCR reagent containing lyophilized excipients for the detection of RNA templates that can be lyophilized with a suitable lyophilization process. The difficulties in the development of lyophilized products include three aspects: 1. The removal of liquid reagents affects the lyophilized components, and the stability is poor; 2. It is difficult to freeze-dry and mold, and the yield rate is low; 3. Poor long-term storage stability of lyophilized finished products. Through the optimization of functional proteins, the matching and screening of excipients and lyophilization processes, this product successfully solves the above three problems. Through the screening of enzymes and excipients with high stability, the storage stability of this product is consistent with that of glycerin-containing products; By selecting the appropriate lyophilized excipients and matching the lyophilization process, the yield and stability of the lyophilized products are improved, so that the lyophilized products can be stored at room temperature for more than one year. In addition, this product offers the advantages of conventional

glycerol reagents, including good multiplex amplification performance, high specificity, and high sensitivity. The dUTP/UDG anti-contamination system is incorporated into the reagents, which can be used at room temperature to eliminate the influence of amplification product contamination on qPCR results and ensure the accuracy of results.

Protocol

1. Preparation of the Lyophilization Reaction System

Reaction Volume	20 μ l	
Lyophilization Volume	12 μ l ^[1]	20 μ l ^[1]
5X GDSlyo One-step Buffer	4 μ l	4 μ l
GDSlyo One-step Enzyme Mix	1 μ l	1 μ l
3X Excipient ^[3]	4 μ l ^[1]	6.7 μ l ^[1]
Primer Forward (10 μ M)	0.4 μ l	0.4 μ l
Primer Reverse (10 μ M)	0.4 μ l	0.4 μ l
TaqMan Probe (10 μ M)	0.2 μ l	0.2 μ l
RNase-free ddH ₂ O	To 12 μ l	To 20 μ l

The amount of each component in the reaction system can be adjusted according to the following principles:

[1] The lyophilization volume can be adjusted according to the needs to ensure that the final concentration of the lyophilized excipients in the lyophilized volume is 1X.

[2] The optimal range for primers is 0.1~1.0 μ M. In general, the primers with a final concentration of 0.2 μ M work well.

[3] The optimal range for probes is 50-250 nM.

[4] The length of the amplification product should be in the range of 80-200 bp.

2. Lyophilization Procedure

Step	Temperature	Time	Vacuum(Pa)
Warehousing	4°C *	-	-
Frozen	4°C	30 min	-
	-40°C	120 min	-
One sublimation	-30°C	600 min	10
Resolve drying	25°C	360 min	10
	40°C	360 min	0

This procedure is suitable for eight rows of lyophilization in place volumes from 12 to 20 μ l, and may need to be adjusted for lyophilized beads or larger volumes.

Lyophilizers vary from manufacturer to manufacturer and model and may require optimization of the lyophilization program.

3. Reconstitute the lyophilized sample

Add template to the lyophilized product, and add RNase-free ddH₂O to 20 μ l, mix and centrifuge well, and perform amplification.

4. Reaction Procedure**Standard RT-qPCR mode (maximum amplification sensitivity) :**

Reverse transcription	55°C ^a	15 min	/
Initial denaturation	95°C	30 sec	/
Circular reaction	95°C	10 sec	45 Cycles
	60°C	30 sec	

Fast RT-qPCR mode (for most applications) ^b:

Reverse transcription	55°C ^a	5 min	/
Initial denaturation	95°C	30 sec	/
Circular reaction	95°C	5 sec	40 Cycles
	60°C	15 sec	

a. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55°C is conducive to improving amplification efficiency and sensitivity.

b. The reaction time and ramp rate of each stage in the fast program can be adjusted according to the actual use of the Real Time PCR instrument and your own needs.

Product Use Limitations

This product is sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.