SYBR Green Dye-Based One-Step qRT-PCR Kit Cat. # SRP128

Storage: Store at -20℃ and protect from light for 1 year. After thawing, store at 4℃ and protect from light for 6 months. Ship at 4℃.

I. Description

The SYBR Green dye-based one-step qRT-PCR kit is ready-to-use 2x concentrated solution for quantitative, reverse transcription-qPCR. Except DNA template and primers, the 2x SYBR Green dye-based one-step qRT-PCR master mix contains all other components for RT and qPCR, including dNTP/dUTP mix, MgCl2, reaction buffer, 4G Reverse Transcriptase, murine RNase inhibitor, heat-labile UDG, and antibody-based hot start Taq DNA polymerase at optimal concentrations for consistent and efficient PCR amplification. For a 20 ul reaction, simply add 10 ul of 2x SYBR Green dye-based one-step qRT-PCR master mix to specific primers, DNA template, 50x ROX reference dye 1 and 2, and water.

This kit is specially designed for SYBR Green I-based qPCR detection using RNA (e.g. SARS-COV-2 virus RNA) as template. Using gene specific primers (GSP), the reverse transcription and PCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination. This kit integrates the superior performance of 4G Reverse Transcriptase and antibody-based hot start Taq DNA Polymerase with optimized buffer, which enables the detection sensitivity of one step qRT-PCR SYBR green kit to reach 1 pg total RNA. The enhancement factor added in the buffer system can effectively reduce the formation of primer dimer and improve the specificity of products. This kit provides a convenient master mix form for easy and quick operation.

Optimal template and primer concentrations, and qPCR program parameters should be determined experimentally by the investigator.

Product Content:
2 × One Step SYBR Green Mix: 1.25 ml / vial (2 vials)
One Step SYBR Green Enzyme Mix: 0.25 ml
50 x ROX Reference Dye 1: 100 µl
50 x ROX Reference Dye 2: 100 µl
RNase-free ddH_2O: 1.25 ml / vial (2 vials)

II. APPLICATIONS: Detection of various RNA nucleic acids of animals, plants, and microorganisms including viruses. This product is for research use only.

III. GENERAL PCR PROTOCOL USING SYBR GREEN DYE-BASED ONE-STEP QRT-PCR KIT

1. Preparation of reaction solution.

Table 1. Set up 20 µl PCR solution in a thin-wall PCR tube on ice by the following recipe

Reagent	20 µl PCR reaction	Final Concentration
2 × One Step SYBR Green Mix	10.0 µl	1×
One Step SYBR Green Enzyme Mix	1.0 µl	1 x
50 x ROX Reference Dye 1 or 2	0.4 µl	1 x
Primer 1 (10 µM)	0.4 µl	0.2 µM
Primer 2 (10 µM)	0.4 µl	0.2 µM
Template RNA	x µl	Total RNA: 1 pg – 1 µg
RNase-free ddH2O	to a total volume of 20.0 µl	

Note:

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

- Generally, the final concentration of primer in the reaction system is 0.2 µM to obtain better amplification effect. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1 - 1.0 µM.
- Due to the high sensitivity of qPCR, the accuracy of template volume has a significant impact on qPCR results. In order to effectively improve the repeatability of the experiment, it is recommended to dilute the template and pipet larger volume (e.g. 2-5 µl/sample) to the reaction system.
- The size of the amplified products should be within the range of 100 – 200 bp.
- 50 x ROX Reference Dye 1 and 2 are used to rectify the error of fluorescence signals between different wells. 50 x ROX Reference Dye 1 is used for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; 50 x ROX Reference Dye 2 is used for ABI 7500, 7500 Fast Real-Time PCR System, and Stratagene Mx3000P. Do not use ROX for Roche and Bio-Rad Real-Time PCR instruments.

2. Program PCR cycler as the following:

Reverse Transcription[a,b]:	50°C for 3 minutes
Initial denaturation[c]:	95°C for 30 seconds
Then 40 cycles of	
Denaturation:	95°C for 10 seconds
Extension[d]:	60°C for 30 seconds
Melting Curve	
	95°C for 15 seconds
	60°C for 60 seconds
	95°C for 15 seconds

Note:

a. For templates with complex secondary structure or high GC content, the reverse transcription temperature can be increased to 55°C, which will improve the sensitivity and performance.
b. Reverse transcription can be extended to 15 min, which will increase the yield of cDNA.
c. Initial denaturation condition is suitable for most amplification reactions. If the template structure is complex, the initial denaturation time can be extended to 3 minutes to improve the initial denaturation effect.
d. The extension time varies between different qPCR instruments used. For ABI 7700 and 7900HT, the extension time should be ≥30 seconds; for ABI 7000 and 7300, the extension time should be ≥31 seconds, and for ABI 7500, ≥34 seconds.
e. Different qPCR instrument types of melting curve acquisition procedures are not the same, and please select the instrument default melting curve acquisition procedures.

IV. KEY FEATURES

* Using gene specific primers (GSP), the reverse transcription and qPCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination.
* High detection sensitivity of one step qRT-PCR SYBR green kit: 1 pg total RNA.
* More stable amplification curve with low concentration template.

Notes For research use only (RUO). Not for use in diagnostic procedures.

1. If white precipitate is found in the Master Mix after thawing, please place it at room temperature for a short while and invert it upside down several times to dissolve the precipitate before use.
2. Avoid repeated freezing and thawing, so as not to cause the decrease of enzyme activity. If the amount of each use is small, it is recommended to aliquot it to small portions.
3. Please invert the Master Mix upside down several times to mix thoroughly. Do not vortex to avoid air bubbles, which will affect the quantitative results. The Master Mix is ready to use after mixing and centrifuging briefly. Mix gently by pipetting. If the air bubbles are generated, please centrifuge again before use.
4. Detection sensitivity of this kit is very high. It is easy to be contaminated by aerosols in the air. Therefore, the preparation of the reaction system should be carried out on clean bench. Sterile tips and reaction tubes should be used in the preparation process. If laboratory conditions permit, special pipettes and tips with filtering element are recommended.
5.

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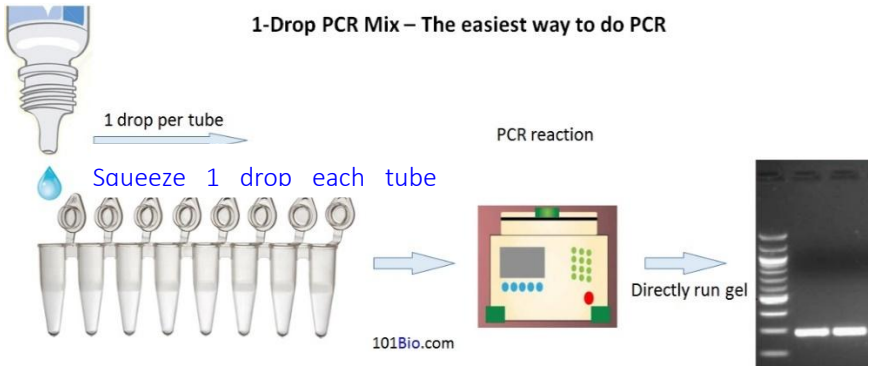
We offer good kits for rapid mouse genotyping as below.

① Mouse Tail DNA extraction kit, Crude Lysis. Cat. # T605 / Fisher Sci. # NC1596514

② Mouse Tail Genomic DNA Kit, Purified Lysis Cat. # W2094

③ Squeeze-1-Drop Do PCR Master mix Cat. # 2599-5 / Fisher # NC1100436

PCR Mix in dripping bottle



101Bio best-selling products.

Lentivirus / Retrovirus	Virus Concentration Solution Cat. # P904C FisherSci # NC3242189
10x Virus Titer-Up Booster	Increases virus titer up to 10-folds # P906 / P909 / Fisher # NC1792390
Lentiviral Expression Vectors	4 promoters: SFFV, CAG, CMV, EF1. 4 selections
Water Bath / Tub Disinfection	1 tablet cleans for 1 week / treats 1-4 gallons. Cat. # T20
Better than Matrigel	Real 3D Cell Culture Gel, 3-stiffness for different cells # P720
Cell Immortalization	SV40T / hTERT Cell Immortalization Kits. Simple & Reliable
Plasmid Miniprep	Endotoxin-Free Plasmid Miniprep Kit Cat. # W2106-50

Mycoplasma Detection qPCR	Mycoplasma causes contamination in cell culture. cat. # T42030
RT-PCR Enzymes / Mix	Make RNA analysis as easy as DNA # W140, 143, 145…