



2×SYBR Real-Time RT-PCR Premixture

Cat. #: PR7102 200 reactions

Storage: -20°C

Description

This kit contains RT reaction reagents and PCR reaction reagents. cDNA is synthesized from template RNA by M-MLV. PCR is performed by using cDNA as template. 2×SYBR real-time PCR premixture is a 2× concentration of premix reagent including *DNA polymerase* and SYBR Green I, specially designed for real-time PCR with intercalator method. This product provides a newly developed buffer, which provides superior specificity, increased amplification efficiency, and high aptitude for high-speed real time PCR. Accordingly, a successful real time PCR is promised with high sensitivity, wide dynamic range, and accurate quantification.

Component

RT reaction reagents

Product	PR7102 (200 reactions)
M-MLV Reverse Transcriptase (200 U/μl)	200 μl
5× First-strand Buffer	1 ml
dNTP Mixture (10 mM each)	200 μl
RNase Inhibitor (40 U/μl)	200 μl
Oligo (dT) ₁₈ Primer (50 μM)	200 μl
RNase-free H ₂ O	5 ml

PCR reaction reagents

Product	PR7102 (200 reactions)
2× SYBR Premixture	1 ml × 5

Procedure

1. Prepare the reverse transcript reacting solution:

Total RNA or Poly (A) RNA	0.2-2 µg
Oligo (dT) ₁₈ (50 µM)	1 µl
dNTP Mixture (10 mM each)	1 µl
RNase-free H ₂ O	Up to 14 µl

2. Incubate on thermal cycler at 65°C for 5 min, then fast chill on ice.

3. Add the reverse transcript reaction solution into above PCR tube:

5× First-strand Buffer	4 µl
M-MLV Reverse Transcriptase (200 U/µl)	1 µl
RNase Inhibitor (40 U/µl)	1 µl
Total	20 µl

4. Reverse transcript reaction on thermal cycler by next conditions.

30°C 10 min

42°C 30-60 min

95°C 5 min

5. Real-time PCR

Reaction Setup:

Component	20 µl	25 µl	50 µl	Final Conc.
2× Premix	10 µl	12.5 µl	25 µl	1×
Upstream Primer				100-400 nm
Downstream Primer				100-400 nm
Template				Pg-ng
Sterile Water	Up to 20 µl	Up to 25 µl	Up to 50 µl	

Thermal Cycler conditions:

Two-step PCR

Cycle numbers	Step	Temperature	Time	Detection
1	1	95°C	2 min	off
40	1	95°C	15 sec	off
	2	60-68°C	20-60 sec	on

Three-step PCR

Cycle numbers	Step	Temperature	Time	Detection
1	1	95°C	2 min	off
40	1	95°C	15 sec	off
	2	55-65°C	10-20 sec	off
	3	72°C	20-60 sec	on

Note:

1. The solution used for cDNA synthesis must be treated by DEPC and can only be used after autoclaving. If some solutions can't be autoclaved, sterilize the solutions by filtration.
2. RNA sample should be avoided from genome contamination.
3. All components should be stored at **-20°C**.
4. Avoid repeatedly freezing and thawing of the reagent.
5. ROX, as the passive dye, provides an internal reference to normalize non-PCR-related fluctuations in fluorescence (e.g. pipetting errors) and machine "noise". ROX also provides a stable baseline for qPCR. ROX, which is not included in this kit, is not necessary for experiments if application plots and dissociation curve are ideal after optimizing reaction system and thermal program.
6. **Signalway's real-time qPCR products are universal reagents that can be used in any real-time thermal cyclers**, such as ABI PRISM7000/7700/7900HT, 7300/7500 Real-time PCR system, 7500 Fast Real-time PCR System (Applied Biosystems), ABI GeneAmp5700, StratageneMx3000, Mx3005P, Mx4000, and Bio-Rad instruments.