Anigen SYBR green RT-PCR Kit

■ Principles

Anigen SYBR green RT-PCR Kit is designed for easy and accurate real-time quantification of RNA from PCR sample. SYBR green I mixed master mix can detect PCR products without target specific probe.

Anigen SYBR green RT-PCR Kit uses Hot Start Taq DNA polymerase, and the RT-PCR buffering conditions are finely adjusted to provide optimum results. ROX reference dye is optional for selection according to Real –Time PCR apparatuses.

The SYBR green I binds to double stranded DNA as a fluorescent dye, and the melting curve analysis is shown specific amplification of the target.

■ Materials provided

1) 2x SYBR green RT-PCR master mix 25ml
: Hot start Taq DNA polymerase, SYBR green Master mix buffer, dNTP mix, SYBR green I, ROX reference dye, $MgCl_2$
2) RT-mix.....500 ㎕
3) ROX reference dye*.....1ml
4) RNase free water.....20ml
5) Insert.....1 EA

* ROX Reference Dye: ROX reference Dye can be included in the reaction to normalize the fluorescent reporter signal, for instruments that are compatible with that option. ROX is supplied at a 25μM concentration, and is composed of a glycine conjugate of 5-Carboxy-rhodamine, succinimidyl ester in 20 mM Tris-HCl (pH 8.4), 0.1mM EDTA, and 0.01% Tween 20. Use the following table to determine the amount of ROX to use with a particular instrument:

Instrument	Amount of ROX per 50㎕ reaction	Final ROX Concentration
ABI 7000, 7300, 7900HT, and 7900 HT Fast	1㎕	500nM
ABI 7500; Stratagene Mx3000™, MX3005P™, and MX4000™	0.1㎕	50nM

-To accurately pipette 0.1㎕ per reaction, we recommend that you dilute ROX 1:10 immediately before use and use 1㎕ of the dilution.

■ Storage and Stability

Store at -20℃. 2x SYBR green RT-PCR master mix should be protected from light.

■ Procedure

1) Thaw each reagent in ice.
2) Prepare PCR reaction mixture as below

Components	Volume/reaction	Final concentration
2x SYBR green RT-PCR master mix	25㎕	1x
Primer A	Variable	0.5μM
Primer B	Variable	0.5μM
RT-mix	1㎕	
Template RNA	Variable	≤500ng/reaction
RNase free water	Variable	
ROX reference dye	1㎕	
Optional: Uracil-N-glycosylase	Variable	1~2units/reaction
Total reaction volume	50㎕	

* Add the Template RNA at 4 step.

3) Mix the PCR reaction mixture completely, and dispense PCR tubes or Plate wells.
4) Add the Template RNA to the individual tube or well.

5) Set up the Real –Time PCR cycler as below.

Step		Time	Tem.	Additional Comments
Reverse transcription		30min	42℃	cDNA synthesis step
PCR initial activation step		15min	95℃	Hot Start Taq DNA Polymerase is activated by this heating step
3-step cycling	Denaturation	15sec	94℃	
	Annealing	30sec	50~60℃	Approximately 5~8℃ below T_m of primers
	Extension	30sec	72℃	Perform fluorescence data collection, unless an additional data acquisition step has been intergraded
	Number of cycle	35~45cycle		Number of cycles depends on the amount of template RNA and transcript abundance

6) Place the PCR tubes or Plate in the Real-time PCR cycler and start the program.
7) Perform the melting curve analysis.
8) Optional: Confirm the amplification using electrophoresis for Specificity of Real-Time PCR.

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