SYBR Green qRT-PCR Master Mix

Code No.:QSR-200 Volume:0.5ml Mix ×5;0.5ml Mn2+

DESCRIPTION

This product is a 2 × Master Mix for "one step qRT-PCR" using hot start rTth DNA polymerase , which exhibits reverse transcriptase activity in the presence of Mn2+ ions. The master mix with Mn2+ allows for " one step qRT-PCR ", including reverse transcription and qPCR steps. This reagent can be applied to an intercalation assay with SYBR Green I.

STORAGE

This reagent can be stored at 4°C for 2 months and protected from light.
For longer storage, this reagent should be kept at -20°C and protected from light.

COMPONENTS

This reagent includes the following components for 100 reactionswith a total of 50ul per reaction, or for 250reactionswith a total of 20ul per reaction.

<QSR-200>

SYBRGreen qRT-PCR Master Mix	0.5mlx5tube
50mM Mn(OAc)2	0.5mlx1tube

DETECTION

This reagent can be used in general detection devices, not needing ROX such as:

-LineGene(bioer);LightCycler (Roche); iCycler iQ, CFX 96(Biorad/MJ);Thermal Cycler Dice(Takara);

This reagent with 1×ROX can be used in detection equipment using passive reference, such as:

-ABI PRISM® 7000, 7700, 7900 ,7300;Step One plus, Step one, ABI PRISM® 7500, 7500Fast(ABI);etc.

PRIMER DESIGN

-Primer length: 20~30 mer
-GC content of primer: 40~60%
-Target length: ≤ 200 bp (optimally, ≤ 150bp)

SPECIMEN

Total RNA: Total RNA typically contains 1~2% mRNA, which can be used as template directly with this kit. Total RNA can be prepared by trizol or the spin column method contains genomic DNA; the total RNA should be treated with DNase I prior to reverse transcription.
Poly(A)+ RNA :Poly(A)+RNA can be used to detect low-level expressing mRNA. However, poly(A)+RNA should be treated carefully , because Poly (A)+ RNA is more sensitive to RNase than total RNA.

PROTOCOL

- All solutions should be thawed on ice, gently vortexed and briefly centrifuged.
- Prepare the following reaction in a thin-walled qPCR tube or plate on ice:

For a total 50 µl reaction volume

Component	Volume	Final concentration
SYBR Green qRT-PCR Master Mix	25ul	1X
50mM Mn(OAc)2	2.5 ul	2.5mM
10uM Forward primer	1ul	0.2uM
10uM Reverse primer	1ul	0.2uM
Template RNA	5ul	Total RNA<2.5ug Poly (A)+ RNA<500ng
PCR grade water	15.5 ul	
Total Volume	50ul	

For a total 20 µl reaction volume

Component	Volume	Final concentration
SYBR Green qRT-PCR Master Mix	10ul	1X
50mM Mn(OAc)2	1ul	2.5mM
10uM Forward primer	0.4ul	0.2uM
10uM Reverse primer	0.4ul	0.2uM
Template RNA	2ul	Total RNA< 1ug Poly (A)+ RNA<200ng
PCR grade water	6.2ul	
Total Volume	20ul	

- Gently mix the reaction solutions and spin down in microcentrifuge.

CYCLING CONDITIONS

[3-Step Cycle]:

Denaturation :	90 °C, 30 sec.
Reverse transcription :	61 °C, 20 min.
Pre-denaturation :	95 °C, 1 min.
Denaturation :	95 °C, 15 sec.
Annealing :	55-65 °C, 15 sec.
Extension :	74 °C, 45 sec

45 cycles

(data collection)

Melting curve analysis

NOTES

-Primer concentrations can be further optimized, if needed. The optimal range of primers is 0.2~0.6 uM. In the case of commercially available primers those recommended condition should be used.
-The final concentration of Mn(OAc)2 should be adjusted to 2~3.5 mM.
-The first denaturation step 30 sec. is sufficient to inactivate the anti-tTh antibodies. Do not prolong this denaturation step.
-The annealing temperature should to Tm - 5 °C, and the optimal range is 55-65°C.
-The temperature transition rate could be set to 20°C/sec. Pool amplification may be improved by adjusting the temperature transition rate to 2°C/sec
-If the target length is ≤ 200 bp, the extension time should be adjusted to 15 sec. Data collection steps should be at least 15 sec.