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Enseignement et Recherche

TECHNICAL SHEET:

2×TaqMan PCR MasterMix (Probe qPCR)

Ref : PW06-01

Specification: 50T

Storage: Store at a constant temperature of -20°C, valid for one year.

Product Description:

2×Taqman PCR MasterMix(Probe qPCR) is a kind of specific reagent for real-time PCR by probe. It can totally blocked Taq enzymatic activity at RT to start HS taq DNA polymerase, can inhabit the extension of nonspecific primers and the formation of primer dimmers at low temperature. The reagent adopts qPCR specific buffer, which can improve the efficiency and sensitivity of qPCR. Standard curve can be obtained in a wider quantification area.The reagent is compatible with many factories, for example,Applied Biosystems, Eppendorf, Bio-Rad and Roche.

Steps:

Standard operating procedure:

1. Prepare the reaction system according to the following table

Component	25 µl Volume	50 µl Volume	Final concentration
2×TaqMan PCR MasterMix	12.5 µl	25 µl	1 x
Primer 1 (10 µM)	0.5 – 2.5 µl	1 – 5 µl	0.2 ~ 1.0 µM
Primer 2 (10 µM)	0.5 – 2.5 µl	1 – 5 µl	0.2 ~ 1.0 µM
TaqMan Probe	1 µl	2 µl	-
Template DNA	5 µl	10 µl	-
ddH2O	-	-	-
Total Volume	25 µl	50 µl	-

1. In general, the final concentration of primer is 2 µM, if the result is not ideal please optimize the concentration from 0.2 to 1.0µM .
2. The optimized probe concentration is 0.1-0.3µM. It is necessary to determine optimal conditions for the experiment. The use of probe is related to real time PCR amplicfiers, probe species, fluorescent labeled material species.

Quality Control

1. function test: sensitivity, specific, reproducibility of qPCR.
2. no exogenous ribonuclease activity,endonuclease activity,and no exonucleared deoxyribonuclease contamination.

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PCR Cycling Protocol

- **Two Step Cycling**

Pre-denaturation	Denature	Anneal / Extend
1 CYCLE	35-45 CYCLES	
95°C	95°C	60°C
10 min	10 – 20 S	20 – 60 S

- **Three Step Cycling**

Pre-denaturation	Denature	Anneal	Extend
1 CYCLE	35-45 CYCLES		
95°C	95°C	56 – 64°C	72°C
10 min	10 – 20 S	10 – 30 S	10 – 60 S

Note :

1- For Roche Light Cycler480, the pre-denaturation time suggest 10min, ABI7500 suggest 5mins.
2- If the annealing temperature is lower or exceed 200bp, please use the three-step method.
3- Use Special areas and pipettes before and after amplification. Wear gloves and change them frequently. Do not open the reaction tube after the completion of PCR reaction to minimize contamination of PCR products to samples