template can be adjusted accordingly, but can't exceed 10ul.

4. Do brevity centrifuging for PCR reaction tubes to ensure all liquid stay at the bottom of tube.

5. PCR reaction

Real-Time PCR reaction's program is set as following:

Tow Step reaction is set as following: 94℃，2min； 94℃，10s； 60℃，40s；　　40cycles Fluorescence signal adopting is set to 60℃(the second step in each circle)；

Adjust plus to make fluorescence base as 20-30，may adjust according to reagent's situation; Select suitable detection channel accords fluorescence probe.

Note: Annealing and extension temperature 60 ℃ can do inching according to the Tm value of primer and probe.

➢ Result analysis and determinant

1 Input concentration of standard or frame of reference, select "Fit Points" method for analysis. Select a comparative smooth sect (without abnormal fluctuation) of fluorescence signal before the inflexion as base line adjustment. The threshold is the highest point where the base line does exceed normal negative contrast curve (ruleless noise line), and then do qPCR analysis. You may adjust according to instrument's noise state.

2 Record unknown sample's concentration or CT value after analysis.

3 Also could use gel electrophoresis to check and analyze the specificity of PCR product.

➢ Notices

1 Please read this users' manual carefully before doing experiment；

2 This kit is just for research use, the result is just for reference;

3 Please shorten the time of placing the reagents under room temperature as possible, and store them under -20℃ immediately;

4 It's possible to be polluted for nucleic acid expansion. So please accords to standard PCR operation instruction strictly.

➢ Transport and storage condition

Stored at -15℃～-20℃.

BioEasy Taqman PCR Magic Mix

(Cat NO. BSB08L1)

Users Manual

(200T)

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Tel: 0571-87774567　　**Fax:** 0571-87774556

Web: www.bioer.com.cn　　**Email:** reagent@bioer.com.cn

➢ Introduction

This product is a standard reagent that is designed for the technology of Taqman probe. User can do real-time PCR reaction directly by adding primer, template and Taqman probe.

➢ Components

Components	Volume/ul	Quantity
2×Taqman Mix(with 6.0mM Mg^{2+})	1000	4
Taq polymerase	32	2
$MgCl_2$	1000	2
ddH_2O	1000	2

➢ Applied Instrument

Line-Gene series Real-time PCR system from BIOER TECHNOLOGY CO., LTD. or the same type of instrument from other companies.

➢ Quality Control

Suggest taking plasmid DNA as template, expanding at least 4 grads in the Line-Gene series Real-time PCR detection system, the correlation coefficient should be less than or equal to -0.990.

➢ Important Parameters

1. Template

This kit applies to plasmid DNA（10-10^7 copies）, genomic DNA（100pg-1ug）or cDNA(1pg-100ng). To get the best result, the length of expansion segment should be in the bound of 50-200bp.

2. Primer and Probe

Primer and probe are the important parameters in the Real-time PCR reaction. During the time of designing primer and probe, suggest using special software, such as Primer Express, beacon Designer, AlleledID.

Normally, the concentration of primer and probe is adjusting during 0.1uM-1.0uM.

3. Concentration of ion

The PCR reaction liquid in this kit contains Mg^{2+} in end concentration 3mM. This is suitable for normal situation. However, you can adjust the concentration of Mg^{2+} for different intention segment. This kit contains an attaching tube of 25mM $MgCl_2$.

Adjust the concentration of Mg^{2+} according the following list:

End concentration of Mg^{2+}（mM）	Quantity of 25mM $MgCl_2$ added into reaction（ul）
4.0	1.6
5.0	3.2
6.0	4.8

➢ Protocol

1. Take out 2×Taqman Mix、 $MgCl_2$、ddH_2O to ice-out under room temperature and gently reverse up to down, to brevity centrifuging before confecting PCR reaction liquid.
2. Components of PCR reaction liquid（please confect on the ice）

Components	Volume（ul）
2×Taqman Mix ①	20
PCR Forward Primer （10uM）	2.4（Adding by self）
PCR Reverse Primer （10uM）	2.4（Adding by self）
Probe(10uM)	0.8（Adding by self）
Taq polymerase	0.3
ddH_2O	10.1
Template②	4
Total	40

① 2×Taqman Mix contains PCR buffer、 Mg^{2+} 、dNTP mixture, etc.

② If it comes to the pinches, can dilute gradient to ensure the best adding quantity of DNA template, because different kinds of DNA templates contain different copies number of target gene.

③ May adjust primer and probe's concentration according different outcomes.

3. Mix reaction liquid fully, load to each PCR reaction tubes. Add 4ul template-plasmid DNA（10-10^7 copies）, genomic DNA（100pg-1ug） or cDNA(1pg-100ng) into each PCR reaction tubes. The volume added into