4ul 模板---质粒 DNA(10-10^7拷贝)、基因组 DNA(100pg-1ug)或 cDNA(1pg-100ng)。加入模板的体积可作适当调整，但不应超过 10ul。

4　将加好模板的 PCR 反应管进行短暂离心，以确保所有试剂流到管子底部。

5　PCR 反应

Real Time PCR 反应程序设置如下：

三步法反应程序设置如下：	二步法反应程序设置如下：
94℃，2min； 94℃，10s, 60℃，15s, 72℃，30s；40cycles 荧光信号采集设在 72℃(每循环第三步反应时)；	94℃，2min； 94℃，10s, 60℃，30s；　40cycles 荧光信号采集设在 60℃(每循环第二步反应时)；

融解度曲线程序设置如下：

95℃，2min；72℃，1min；

95℃，30s，步进 0.5℃/s；

30℃，1min。

在运行之前，调节增益使荧光本底值≤20，也可根据试剂的实际情况调整；仪器检测通道选择设定为 F1 (SYBR Green I)通道。

注：在进行 PCR 程序选择时，无特殊要求建议采用三步法程序进行扩增。若两步法的扩增结果与三步法的扩增结果一致，也可采用两步法替代三步法，以节约时间。

七、结果分析与判定：

1　将标准品或参照物的浓度输入，选择样点拟合法进行分析，基线（零点调整）根据扩增曲线实际情况选取一段相对平稳（无异常波动）的拐点之前的荧光信号作为基线调整，噪声容限以基线刚好超过正常阴性对照品扩增曲线（无规则的噪音线）的最高点，然后进行定量分析。也可根据仪器噪音情况另行调整。

2　分析后记录未知样品的浓度或 CT 值。

3　融解曲线分析有自动分析和手动分析，根据需要进行选择。分析后记录 Tm 值。

4　也可以选择利用琼脂糖凝胶电泳检查分析 PCR 产物的特异性。

八、试剂盒使用注意事项

1　在实验前，请详细阅读此说明；

2　本试剂盒仅供科研使用，结果仅供参考。

3　融解后的试剂尽可能缩短常温放置时间，使用完毕后请立即保存于-20℃。

4　本试剂中含有荧光染料，保存试剂或配制 PCR 反应液时请避光放置。

5　核酸扩增时容易污染，导致结果不可靠。故要求在进行扩增实验时，严格按照标准 PCR 流程进行。

BioEasy SYBR Green I Real Time PCR Kit Manual

说

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书

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(200T)

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一、 前言

本试剂盒是采用核酸扩增技术结合 SYBR Green I 嵌合荧光法进行 Real Time PCR 的专用试剂，可以快速正确地对目的基因进行检测、定量。本试剂盒提供 2×PCR 反应液，包含了除 Taq 酶与引物之外的其它所有组份。使用时只需加入引物、模板、Taq 酶即可进行 Real Time PCR 反应，操作非常快捷简单。

SYBR Green I 是一种荧光染料，能特异性地掺入到双链 D NA 分子的小沟部位，发射荧光信号。在 PCR 反应体系中，当 SYBR Green I 染料与双链 DNA 结合，在激发光的作用下发出荧光，通过荧光强度的测定，即可确定双链 DNA 的含量。荧光染料的优势在于它能监测任何 dsDNA 序列的扩增，不需要探针的设计，使检测方法变得简便，同时也降低了检测的成本。并且在 PCR 结束后可直接进行融解曲线反应分析。通过融解曲线的分析，能判断是否存在着变异或非特异性的扩增。SYBR Green I 的最大吸收波长约为 497nm，发射波长最大约为 520nm。

二、 试剂盒组成

试剂盒组成	体积/ul	数量
2×SYBR Mix(with 4.0mM Mg^{2+})	1250	4
Taq DNA Polymerase	32	2
25mM $MgCl_2$	1000	2
ddH_2O	1000	4
ROX Reference (50×)	200	1

-20℃ 避光保存，避免反复冻融

三、 适用仪器：

Line-gene 荧光定量 PCR 检测系统及其它同类仪器。

四、 试剂盒质量控制

该产品以质粒 DNA 为模板，在 Line-Gene 荧光定量 PCR 检测系统上扩增，至少扩增四个梯度，其相关系数小于等于-0.980。

五、 重要参数

1 模板

本试剂盒适用的模板为质粒 DNA（10-10^7 拷贝）、基因组 DNA（100pg-1ug）和 cDNA(1pg-100ng)。为得到最好的实验结果，扩增的片段长度应在 80-500bp 范围内。

2 引物

引物是 Real Time PCR 反应的重要参数之一。在引物设计时，建议使用 Oligo、Primer 5 等引物设计软件。在 PCR 反应时，引物终浓度通常在

Important Parameters

Template

Using 10-10^7 copies of plasmid DNA, 100pg to 1ug of genomic DNA or 1pg to 100ng of cDNA. In order to acquire the best experiment result, the amplification length should be approximately 80-500bp.

Primers

Primer design is one of the most important parameters when using SYBR Green detection system. We strongly recommend using primer design software such as Oligo, Primer5. When designing primers, the 0primers length should be approximately 15-40bp. Optimal results may require a titration of primer concentrations between 100 and 500 nM. A final concentration of 200 nM per primer is effective for most reactions.

Magnesium Concentration

$MgCl_2$ is included in the SYBR Mix at a final concentration of 2 mM. This concentration works well for most targets; however, the optimal concentration may range from 2 to 6 mM. If necessary, use the 25 mM $MgCl_2$ provided in the kit to increase the concentration, as shown below.

Final $MgCl_2$ concentration	**Add this volume of $MgCl_2$**
3.0 mM	2 ul
4.0 mM	4 ul
5.0 mM	6 ul
6.0 mM	8 ul

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. Use the following table to determine the amount of Rox Reference Dye to use with a particular instrument:

Intrument	**Amount of ROX Reference Dye（50×）per 50 ul reaction（ul）**
ABI7000，7300，7300，7900HT，7900HT Fast	1
ABI7500,Stratagene Mx3000, Mx3005P, and Mx4000	0.1

Using of the kit

1　Defrosting the reagents（2×SYBR Mix、ddH_2O）in the kit at room temperature. Before preparing PCR reagents, please centrifuge all reagents for a few seconds.

2　Set up reactions as specified below. Volumes for a single 50ul reaction are listed.

Component	Single（ul）
2×SYBR Mix(with 4.0mM Mg^{2+})①	25
PCR Forward Primer （10uM）	1
PCR Reverse Primer （10uM）	1
Taq Polymerase	0.3
ddH_2O	18.7
Template②	4
Total	50

① 2×SYBR Mix includes PCR buffer, Mg^{2+} ,dNTP mixture, SYBR Green I etc.

② According to different copies of target Gene between different kinds of DNA template, if possible user should confirm the additional quantity of DNA template by doing Gradient dilution.

3 After mixing PCR reagents above, distribute them into 0.2ml PCR tubes. Add the samples as per 4-10ul each into PCR tubes above.。

4 PCR Reaction

Program your Real Time PCR as shown below. Optimal temperatures and incubation times may vary.

Three step standard cycling program	Two step standard cycling program
94℃，2min ； 94℃，10s ； 60℃，15s ； 72℃，30s，40cycles Choose F1 channels when collecting fluorescent signals and set fluorescent signals detecting at 72℃	94℃，2min ； 94℃，10s ； 60℃，30s； 40cycles Choose F1 channels when collecting fluorescent signals and set fluorescent signals detecting at 60℃

Before running, please adjust gain to make the F1 (SYBR Green I) background ≤20.

Program your melting curve as shown below.

95℃，2min；
72℃，1min；
95℃，30s，step 0.5℃/s；
30℃，1min。

Note: when user is selecting the PCR programme, the three step methods will be recommended for amplification. If the amplification result of two-step is the same as the one of three-step method, user also can use two-step method instead of three-step method for saving time.

Result analysis and judgments

1 Input the concentrations of four positive References. Select fit point method to analyze. Confirm the base line (zero adjustment) by getting the fluorescent signals.

Make the noise limit just beyond the peak of the amplification curve (rule less noise line) of normal negative control; then do quantitative analysis. You could also adjust by yourself according to the condition of instrument's noises.

2 Please record the concentration or CT value of non-known sample after analysis.

3 There are auto and manual for users choosing in melting curve analysis and record Tm value after analysis.

4 Users also can use agarose gel electrophoresis to analyze specificity of PCR products

Note:

1. Please read this manual carefully before beginning the experiment.
2. Don't touch hand directly when you use one-time consumable such as pipe and centrifuge tube.
3. Be caution during the whole process to ensure the accuracy of the experiment. After finishing the experiment, in order to avoid pollution, please use 75% alcohol to clear the worktable.
4. The melted reagent should be stored as shorten as possible, and store them in environment of -20℃ immediately after testing.
5. Please store reagent or PCR reaction system in shadow place due to the fluorescence dye contained
6. Nucleic acid is easy to be contaminated and resulted in to the trusted result, so user should strictly do the amplification experiment according to standard PCR process.

Sample storage

Please store the sample at -20℃ and avoid repeating frozen.

Sample transportation

Please transport the sample at or lower than 4℃.

TECHNICAL SUPPORT:

For technical support, please dial phone number +86-571-87774567-5211 or 87774575, by fax to +86-571-87774303, or by email to reagent@bioer.com.cn.

CONTACT INFORMATION OF MANUFACTURER.

HANGZHOU BIOER TECHNOLOGY CO.,LTD.
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Description

Adopting nucleic acid amplification technique and combining intermediate fluorescence method, SYBR Green I real-time PCR can do detection and quantification on target gene fast and properly. The kit offers 2×SYBR Mix, including all components except for Taq DNA polymerase and primers. During operation, user can do real-time PCR reaction directly by adding primer, template and Taq DNA polymerase, which is very simple.

SYBR Green I is one kind of fluorescence dye, which will excited fluorescence signal after specially inserted in dsDNA molecule. In PCR reaction system, when SYBR Green I dye combine with dsDNA, and excited with excited light, via testing strength of fluorescence, user can calculate the quantity of dsDNA. The advantage of fluorescence dye is that: it can be used in testing any dsDNA amplification, without design of probe, and make the testing method easier, meanwhile, it can decrease cost, what's more, user can analyze melt curve directly. As soon as the PCR test finish. By the analysis of melt curve, user can judge whether there are existing aberrance or non-specific amplification. The Max Absorb wave length of SYBR Green I is 497nm, the Max. Emission wavelength is about 520nm.

Component

component	size/ul	Quantity
2×SYBR Mix(with 4.0mM Mg^{2+})	1250	4
Taq DNA Polymerase	32	2
25mM $MgCl_2$	1000	2
ddH_2O	1000	4
ROX Reference (50×)	200	1

Instrument

Bioer Line-Gene Real Time PCR Detection System, or the same kind of the instruments in other companies.

Quality Control

This product is tested functionally in Real Time using plasmid DNA. Kinetic analysis must demonstrate a linear dose response with decreasing target concentration over four orders of magnitude, and the relative parameter of standard curve should be ≤-0.980.

0.1uM-0.5uM 之间调整，一般终浓度为 0.2uM 时可得较好的结果。

3 **镁离子浓度**

本试剂盒的 PCR 反应液已含有终浓度为 2.0mM 的镁离子，一般情况即可得较好结果。但对于扩增不同的目的片段，镁离子终浓度可作适当调整。本试剂盒中附送一管 25mM 氯化镁。根据下表使用量，对镁离子终浓度作调整：

镁离子终浓度（mM)	加入 25mM 氯化镁的量（ul）
3.0	2
4.0	4
5.0	6
6.0	8

4 ROX Reference Dye

ROX Reference Dye 在使用 ABI、Stratagene 等公司的荧光定量 PCR 仪上，用以校正孔与孔之间产生的荧光信号误差。具体如下：

仪器	每 50ul 体系加入 ROX Reference Dye（50×）的量（ul）
ABI7000，7300，7300，7900HT，7900HT Fast	1
ABI7500,Stratagene Mx3000, Mx3005P, and Mx4000	0.1

六、使用方法：

1 取出 2×PCR 反应液、ddH_2O,进行室温解冻，上下轻缓颠倒混匀，在配制前可进行短暂离心。

2 PCR 反应液配制组分：（反应液配制时请在冰上进行）

试剂组分	体积（ul）
2×SYBR Mix(with 4.0mM Mg^{2+})①	25
PCR Forward Primer （10uM）	1
PCR Reverse Primer （10uM）	1
Taq DNA Polymerase	0.3
ddH_2O	18.7
模板②	4
Total	50

① PCR 反应液内含 PCR buffer、 Mg^{2+}、dNTP mixture、SYBR Green I 等。

② 因不同种类的 DNA 模板中含有的靶基因的拷贝数不同，必要时可进行梯度稀释确定最佳的 DNA 模板添加量。

3 充分混匀反应液，分装至各个 PCR 反应管中。在每个 PCR 反应管中，各加入