



2x Realtime PCR Super mix (SYBRgreen,with anti-Taq) 使用说明书

| 产品名称 | 单位 | 货号 |
|--------------------------|--------------|----------|
| 2×Realtime PCR Super mix | 1ml*5 支 | S6013-01 |
| 2×Realtime PCR Super mix | (1ml*5 支)×5 | S6013-05 |
| 2×Realtime PCR Super mix | (1ml*5 支)×10 | S6013-10 |

【储存条件】

请置于-20℃保存，有效期24个月。经常使用，可置于4℃保存，效期六个月。

【产品简介】

本产品是采用 SYBR Green I 嵌合荧光法进行实时荧光定量 PCR 的专用 2×浓度预混液。利用 HotStart Taq DNA Polymerase 高温加热前，anti-Taq 单克隆抗体与 Taq 酶结合，抑制 Taq 酶的聚合酶活性，从而抑制在低温条件下出现的由引物和模板 DNA 非特异性杂交或引物二聚体引起的非特异性扩增。Anti-Taq 单克隆抗体在 PCR 反应第一循环的变性步骤中已完全失活，不会阻碍之后的 Taq Polymerase 反应，大大提高了 PCR 反应的灵敏度及特异性。优化浓度的 SYBR Green I 荧光染料，特异性地掺入 DNA 双链后，荧光信号增强，而没有掺入链中的 SYBR Green I 染料分子的荧光信号不变，从而保证荧光信号的增加与 PCR 产物的增加完全同步，荧光可以在退火或延伸阶段测定。

【产品组份】

HotStart Taq DNA Polymerase、SYBR Green I、dNTPs、Mg²⁺、ROX、反应缓冲液、稳定剂和增强剂。

【适用范围】

主要用于基因组 DNA 靶序列和 RNA 反转录后 cDNA 靶序列的定量检测。本品具有高通用性，适用于各种仪器。因产品中已经添加了 ROX Reference Dye，因此可以用于需要校正荧光信号的仪器（如 ABI Prism7000/7300/7900HT 和 ABI Step One /ABI Step One Plus 荧光定量 PCR 仪）。也可以用于 Stratagene、Roche、Bio-RAD 和 Eppendorf 等各种荧光定量 PCR 仪上采用 SYBRGreen 法进行基因表达分析和核酸检测等实验。

【所需试剂】

本产品为 2×预混荧光定量 PCR 反应体系，使用时只需加入模板、引物和水，使其工作浓度为 1×，即可进行反应。具有快速简便、灵敏度高、特异性强、稳定性好等优点，可最大限度地减少人为误差、节约 PCR 实验操作时间、降低污染几率。

【操作示例】

| 建议的 PCR 条件: | | 按下表配制PCR 反应体系: | |
|--|--------|---|-------------|
| Template DNA | X*μl | 95℃ | 30-60 sec. |
| 2×Realtime PCR Super mix Primer 1 (10μM) | 10 μl | 35-40 cycles of: 95℃ | 15 sec. |
| Primer 2 (10μM) | 0.5 μl | 55-65℃ | 15 sec. |
| ddH2O 补足至 | 0.5 μl | 72℃ | 30-60 sec*. |
| | 20 μl | *一般情况下目标片段在 300bp 以下时，延伸时间 30 秒即可，但一部分仪器，为测定稳定的荧光，延伸时间需要大于 30 秒。扩增曲线散乱，或者各孔间差异较大时，请设定较长的延伸时间（45-60 秒） | |

*:10~100 ng 基因组 DNA，或 1~10 ng cDNA 为参照，因不同物种的模板中含有的目的基因拷贝数不同，可对模板进行梯度稀释，以确定最佳的模板使用量。另外 two Step RT PCR 反应的 cDNA (RT 反应液) 作为模板时的添加量不要超过 PCR 反应液总体积的 10%。

【注意事项】

- 使用前请上下颠倒轻轻混匀，尽量避免起泡，并经短暂离心后使用。
- 本品对光敏感，尽量减少光线暴露时间，长时间的曝光会导致荧光信号减弱或丧失。本品不能用于杂交探针法。

2x Realtime PCR Super mix (SYBRgreen, with anti-Taq) User Manual

| Product | Units | Cat.# |
|--------------------------|-----------------|----------|
| 2×Realtime PCR Super mix | 1ml*5tubs | S6013-01 |
| 2×Realtime PCR Super mix | (1ml*5tubes)×5 | S6013-05 |
| 2×Realtime PCR Super mix | (1ml*5tubes)×10 | S6013-10 |

Store at -20°C, protected from light.

Description

S6013 is a Taq DNA polymerase-based 2x Super mix for real-time PCR, which contains all components, except for the primer. This reagent is applicable for intercalation assay with SYBR® Green I. This reagent can be used in glass capillary systems (e.g., LightCycler, Roche Molecular Systems, Inc.). This reagent can be used in a passive reference system (e.g., ABI PRISM® 7700, Applied Biosystems, Inc.). The passive reference dye does not affect any other systems. Hot Start technology with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

Detection

S6013 can be used in general detection devices, such as: LineGene (Bioer Technology co., Ltd.); S6013 can also be used in detection equipment using glass capillaries or passive reference, such as: LightCycler (Roche Molecular Systems); ABI PRISM® 7000, 7700, and 7900 (Applied Biosystems). Note: The passive reference mode of detectors should be set at "ROX".

Specimen

1. cDNA: Reverse transcription reactions from total or poly (A)+ RNA may be used directly, or after dilution, for real-time PCR. Purified cDNA by phenol/chloroform extraction and ethanol precipitation may also be used. Oligo dT and random primers are suitable for the reverse transcription reaction. Up to 20% of the synthesized cDNA solution from the S6 Super qPCR RT kit

(Code NO. S6012) may be added to the PCR reaction solution directly, without purification.

2. Genomic DNA: Purified DNA, which would be used for general PCR, is also suitable for real-time PCR. In the case of mammalian genomic DNA, 1~10 ng genomic DNA is sufficient for real-time PCR.

Protocol I

Intercalation assay protocol using ABI PRISM® 7700

The following is an intercalator assay protocol to be used with ABI PRISM® 7700. For other detection devices, this protocol may require modification depending on each instruction manual.

1. Preparation of reaction solution

| Component | Volume | Final Concentration |
|--|------------|---------------------|
| PCR grade water | 16 μ l | |
| Realtime PCR Super Mix | 25 μ l | 1x |
| 10pmol/ μ l (10 μ M) Primer #1 | 2 μ l | 0.4 μ M |
| 10pmol/ μ l (10 μ M) Primer #2 | 2 μ l | 0.4 μ M |
| Template DNA | 5 μ l | |
| Total volume | 50 μ l | |

Notes: The primer concentration can be further optimized, if needed. The optimal range for the primers is 0.2~0.6 μ M. In the case of commercially available primers, recommended conditions from those companies should be used.

2. Cycling conditions <3-step cycle> The following condition is recommended:

| | | |
|-------------------|---------------------------------|----------------|
| Pre-denaturation: | 95°C, 30sec. ~1 min. | |
| Denaturation: | 95°C, 15 sec. | |
| Annealing: | 55~65°C, 15 sec. | |
| Extension: | 72°C, 45 sec. (data collection) | (35~40 cycles) |

Melting curve analysis

Notes:

A. The annealing temperature in 3-step cycle should be set to 55~65°C, depending of the primer T_m value.

B. The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Fifteen seconds is also sufficient for denaturation during each cycle.

C. Data collection step should be longer than 30 sec.

Protocol II Intercalation assay protocol using Roche LightCycler™

The following is an intercalator assay protocol to be used with the Roche LightCycler™. In the case of other detection devices, this protocol should be modified accordingly.

A. Preparation of reaction solution

| Component | Volume | Final Concentration |
|--|-------------|---------------------|
| PCR grade water | 6.4 μ l | |
| Realtime PCR Super Mix | 10 μ l | 1x |
| 10 μ mol/ μ l (10 μ M) Primer #1 | 0.8 μ l | 0.4 μ M |
| 10 μ mol/ μ l (10 μ M) Primer #2 | 0.8 μ l | 0.4 μ M |
| Template DNA | 2 μ l | |
| Total volume | 20 μ l | |

Notes: The primer concentration can be further optimized, if needed. The optimal range for primers is 0.2~0.6 μ M. In the case of commercially available primers, recommended conditions from each manual should be followed.

B. Cycling conditions <3-step cycle> The following condition is recommended:

| | | |
|-------------------|---------------------------------|----------------|
| Pre-denaturation: | 95°C, 30 sec. ~1 min. | |
| Denaturation: | 95°C, 5 sec. | |
| Annealing: | 55~65°C, 10 sec. | |
| Extension: | 72°C, 15 sec. (data collection) | (35~40 cycles) |

Melting curve analysis

Notes:

- The annealing temperature can be set to 55~65°C, depending on the primer T_m value.
- The annealing time should be set for 5~20 seconds. Longer annealing time results in increased efficiency, and a shorter time decreases non-specific amplification.
- The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Five seconds is also sufficient for denaturation during each cycle.
- Data collection step should be longer than 10 sec. If commercially available primers or probes are employed, the recommended conditions from each company should be used.

Science Tool