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	MF013-01
2×Realtime PCR Super mix	(1ml*5 支)×5	MF013-05
2×Realtime PCR Super mix	(1ml*5 支)×10	MF013-10

【储存条件】

长期保存，请置于**-20˚C**，有效期 24 个月。经常使用，可置于 4˚C 保存至少六个月。

【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，而不掺入链中 SYBR Green I 染料分子荧光信号不变，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、Mg^{2+}、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，可用于各种仪器。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 Prism7000/7300/7700/7900HT 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】

按下表配制 PCR 反应体系:

Template DNA	X* µl
2×Realtime PCR Super mix	10 µl
Primer 1 (10µM)	0.5 µl
Primer 2 (10µM)	0.5 µl
ddH_2O 补足至	20 µl

建议的 PCR 条件：

95°C	30-60 sec.
35-40 cycles of:	
95°C	15 sec.
55–65°C	15 sec.
72°C	30-60 sec*.

*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】

1. 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。
2. 尽可能减少在光下的曝露时间，长时间的曝光可导致荧光信号强度的丧失。 本品不能用于杂交探针法。

【备注】

本产品仅供科研使用。在确认产品质量出现问题时，本公司承诺为客户免费更换等量的质量合格产品。

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Store at -20°C, protected from light.

Description

MF013 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

MF013 can be used in general detection devices, such as: LineGene (Bioer Technology co., ltd.); MF013 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 M5 Super qPCR RT kit (Code NO. MF012) 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

1. **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 µl	
Realtime PCR Super Mix	25 µl	1x
10pmol/µl (10 µM) Primer #1	2 µl	0.4 µM
10pmol/µl (10 µM) Primer #2	2 µl	0.4 µM
Template DNA	5 µl	
Total volume	50 µl	

Notes: The primer concentration can be further optimized, if needed. The optimal range for the primers is 0.2~0.6 µM. 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 Tm 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.

2. 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.

(1) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	6.4 µl	
Realtime PCR Super Mix	10 µl	1x
10pmol/µl (10 µM) Primer #1	0.8 µl	0.4 µM
10pmol/µl (10 µM) Primer #2	0.8 µl	0.4 µM
Template DNA	2 µl	
Total volume	20 µl	

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

(2) 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:

A. The annealing temperature can be set to 55~65°C, depending on the primer Tm value.

B. 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.

C. 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.

D. 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.