NOYA-Syber-Detect MMX

Catalog number: N-1141

« Research Use Only »

- Store at -20°C, protected from light

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe
general laboratory precaution and utilize safety while using this kit.

[1] Introduction

Description

This product is a Taq DNA polymerase-based 2 x master mix for real-time PCR, which contains all components, except for the primer. This reagent is applicable for intercalation assay with SYBR® Green I.

Features

-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 enables high specificity and reproducible amplification.

[2] Primer design

Primers should be designed according to the following guidelines:
-Primer length: 20~30 mer
-GC content of primer: 40~60%
-Target length: ≤ 200 bp (optimally, ≤ 150bp)

Notes:
-Longer targets (>200 bp) reduce efficiency and specificity of amplification. The ideal target length range is 50~150 bp.
-Since detection in an intercalation assay is affected by non-specific amplification, primers should be as specific as possible

[3] Detection

This reagent can be used in general detection devices, such as:

-Corbett Real time PCR (Qiagen Co;ltd)
This reagent 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”.

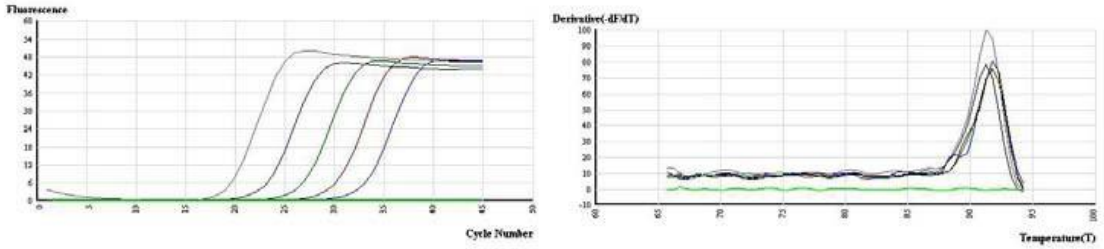

Fig. 1: Amplification curves (left) and melting curves (right) with SYBR® Green Realtime PCR Master Mix

[4] 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 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.

[5] Protocol

Note: Before starting procedure and after 1th thawing, add 7.5µl HS-Enzyme, mix , fuged and keep at-20°C. Avoid more than 2-3 thawing cycle

1. Intercalation assay protocol using ABI PRISM® 7700

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

(i) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	3 µl	
SYBR® Green Realtime PCR Master Mix	10µl	1x
10pmol/µl (10 µM) Primer #1	2 µl	0.4 µM
10pmol/µl (10 µM) Primer #2	2 µl	0.4 µM
Passive Rox(50X)	0.4µl	
Template DNA	3 µl	
Total volume	20 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.

(ii) Cycling condition

The following condition is recommended. Initially, a 3-step cycle should be used.

<3-step cycle>

Pre-denaturation:	95°C, 10 min.	
Denaturation:	95°C, 15 sec.	40 cycles
Annealing**:**	55~65°C, 15 sec.	
Extension:	72°C, 45 sec. (data collection)	

Melting curve:

According to instruments manufacturer recommendation.

Notes:

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

-The pre-denaturation condition described above is sufficient for activationTaq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period

2. Intercalation assay protocol using Roche LightCycler™

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

(i) Preparation of reaction solution

component	volume	Final Concentration
PCR grade water	6.4 µl	
SYBR® Green Realtime PCR Master Mix	10 µl	1 X
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	

Melting curve:

According to instruments manufacturer recommendation.

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.

(ii) Cycling conditions

The following condition is recommended:

<3-step cycle>

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

Notes

-The annealing temperature can be set to 55~65°C, depending on the primer Tm 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 activation of the Taq DNA polymerase 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.

3. Intercalation assay protocol using Corbett Machine

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

(i) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	3 µl	1x
SYBR® Green Realtime PCR Master Mix	10µl	
10pmol/µl (10 µM) Primer #1	2 µl	0.4 µM
10pmol/µl (10 µM) Primer #2	2 µl	0.4 µM
Passive Rox(50X)	0.4µl	
Template DNA	3 µl	
Total volume	20 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.

(ii) Cycling condition

The following condition is recommended. Initially, a 3-step cycle should be used.

<3-step cycle>

Pre-denaturation:	95°C, 10 min.	
Denaturation:	95°C, 15 sec.	40 cycles
Annealing**:**	55~65°C, 20 sec.	
Extension:	72°C, 20 sec. (data collection)	

<2-step cycle>

Pre-denaturation:	95°C, 10 min.	
Denaturation:	95°C, 15 sec	40 cycles
Annealing**:**	60°C, 35 sec. (data collection)	

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[6] Troubleshooting

Symptom	Cause	Solution
No amplification	Incorrect mode setting of detector for the fluorescence dye.	Confirm the detector setting.
	Incorrect setting for data collection.	Confirm the data collection setting.
	Incorrect setting for sample position.	Reposition the sample tubes.
	Inappropriate concentration of primers.	Optimize the concentration of primers according to instructions (see [6]).
	Inappropriate design of primers.	Confirm specificity and Tm value of the primers.
	Inappropriate cycle conditions.	Confirm the primer Tm value. A lower annealing temperature increases amplification efficiency. For GC rich targets, an elongated denaturation time might be effective.
	Low purity or quality of samples	Check the purity or quality of sample DNA.
Variation in detection	Failure or malfunction of the device	Check the device.
	Low quality sample DNA.	Repurify the sample DNA by phenol/chloroform extraction and ethanol precipitation, or other method.
	Inappropriate concentration of primers.	Optimize the concentration of primers according to instructions (see [6]).
	Inappropriate design of primers.	Confirm specificity and Tm values of the primers.
	Inappropriate cycle conditions.	Confirm the Tm values of the primers.
	Variation of dispensed volume.	Increase the reaction volume.
Signals in blank reactions	Contamination of amplicons or sample DNAs.	Use fresh PCR grade water, and remake the primer solution and master mix.
	Detection of non-specific amplification.	Optimize the primer and cycle conditions.