SYBR® Green qPCR Mix (2x)
SAMPLE

Components

Component	NB-03-0117	NB-03-0118
SYBR® Green qPCR Mix (2x)	1 ml	5x 1 ml
Nuclease-free Water	1 ml	5x 1 ml

Storage
This reagent can be stored at 4°C for 2 months and protected from light. For longer storage, this reagent should be kept at -20°C and protected from light.

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.

Application
- 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 with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

Composition of the SYBR Green qPCR Mix

100 mM KCl , 4 mM MgCl2, 400 µM dNTPs, 0.1 U/µl Taq DNA Polymerase, 1x SYBR® Green and other optimized buffer components.

Detection
- This reagent can be used in general detection devices, such as: LineGene (Bioer Technology 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”.

Protocol

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.

1.1 Preparation of reaction solution

Add all the solution in a thin walled PCR tube on ice. For a total 50µl reaction volume

Component of sample	Volume	Final conc.
SYBR Green qPCR Mix (2X)	25 µl	1X
Forward Primer (10 µM)	2 µl	0.4 µM
Reverse Primer(10 µM)	2 µl	0.4 µM
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 50 µl	–

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

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E.coli genomic DNA	10ng-100ng

1.2 Perform PCR using the following thermal cycling conditions

Initial Denaturation	95℃	1 min.
40 Cycles	95℃ 55-65℃ 72℃	15 sec. 15 sec. 45 sec
Melting curve analysis		

2. Intercalation assay protocol using Roche LightCycler™

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

2.1 Preparation of reaction solution

Add all the solution in a thin walled PCR tube on ice. For a total 20µl reaction volume

Component of sample	Volume	Final concentration
SYBR Green qPCR Mix (2X)	10µl	1X
Forward Primer(10 µM)	0.8µl	0.4 µM
Reverse Primer(10 µM)	0.8µ	0.4 µM
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 20 µl	

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

2.2 Perform PCR using the following thermal cycling conditions.

Initial Denaturation	95℃	30 sec.
40 Cycles	95℃ 55-65℃ 72℃	5 sec. 10 sec. 15 sec
Melting curve analysis		

3. One-step RT-PCR by adding a reverse transcriptase

This reagent can be used for a one-step intercalator assay using reverse transcriptase. The following is a one-step protocol using this reagent and a high-efficiency reverse transcriptase.

3.1 Preparation of diluted RTase solution.

Add all the solution in a thin walled PCR tube on ice. (This solution is used in step 2.2.)

Component of sample	Volume	Final conc.
RNasin(40U/µ) (Cat:R2011)	4µl	5U/µl
M-MLV(200U/µ) (Cat:R1041)	0.5µl	3U/µ
Water, nuclease-free	to 33 µl	–

3.2 Preparation of reaction solution.

Component of sample	Volume	Final conc.
SYBR Green qPCR Mix	25lµl	1X
Forward Primer(10 µM)	2µl	0.4 µM
Reverse Primer(10 µM)	2µ	0.4 µM
Diluted M-MLV(3U/µ)(	5µl	0.3U/µl
Template DNA	variable	10 pg-1 µg
Water, nuclease-free	to 50µl	–

Note: Because this method tends to result in non-specific amplifications, the conditions should be optimized. Primer and probe concentrations can be further optimized, if needed. The reverse transcriptase concentration should be decreased when primer dimmers are generated.

3.3 Cycling conditions for ABI PRISM® 7700.

Perform PCR using the following thermal cycling conditions. In the case of other detection devices, this protocol should be modified accordingly.

Reverse transcription	42℃	20 min.
Initial Denaturation	95℃	5 min.
40 Cycles	95℃ 55-65℃ 72℃	15 sec. 15 sec. 45 sec.
Melting curve analysis		

3.4 Cycling conditions for Roche LightCycler™ .

Perform PCR using the following thermal cycling conditions.

Reverse transcription	42℃	20 min.
Initial Denaturation	95℃	5 min.
40 Cycles	95℃ 55-65℃ 72℃	15 sec. 15 sec. 15 sec.
Melting curve analysis		

Guidelines for preventing contamination of PCR reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.