qPCR Hotstart Taq DNA Polymerase

For research use only

Components

Component	P1101 250 U	P1102 1,000 U	P1103 3,000 U	P1104 18,000 U
qPCR Hotstart Taq DNA Polymerase (5 U/μl)	50 μl	200 μl	200 μl × 3	100 μl × 36
10× qPCR Buffer[1]	1.25 ml	1.25 ml × 2	1.25 ml × 6	1.25 ml × 36

[1] qPCR Buffer contains Mg^{2+} and other components that are necessary for qPCR.

Storage

This reagent should be kept at -20°C. The shelf life is 2 years when stored properly.

Description

qPCR Hotstart Taq DNA Polymerase is a hot-start Taq DNA polymerase with antibody modification, and the activity is wholly inhibited at room temperature, which results in less non-specific amplifications. Depending on high temperature to activate the enzyme activity, the reaction has very high specificity and sensitivity. qPCR Hotstart Taq DNA Polymerase adopts advanced production technology, does not introduce contamination of animal-derived DNA. The enzyme has stronger stability, and incomparable advantages over antibody-modified hot-start Taq DNA polymerase. The pre-denaturation time is reduced to 3 minutes, and the amplification efficiency is higher than that of most chemical-modified hot-start DNA polymerase. The elongation rate is 1kb/min. It is a special polymerase for real-time quantitative PCR (qPCR), both dye method and probe method.

Unit Definition

Using activated salmon sperm DNA as template/primer, the activity of ingesting 10 nmol total nucleotides as acid insoluble substances within 30 min at 74°C is defined as 1 unit of activity (U).

Storage Buffer

200mM Tris-HCl, 1mM DTT, 0.1mM EDTA, 100mM KCl, 0.5% Tween20, 50% (V/V) glycerol, 0.5% Triton X -100

10× qPCR Buffer with Mg^{2+}

50mM KCl, 100mM Tris-HCl, 200mM NH_4Cl, 20mM $MgCl_2$

Applications

High-specificity amplification & multiplex qPCR: hot-start polymerase with antibody-like modification
Thermostable: half-life over 40 min at 95°C incubation
Generates 3'-dA overhangs PCR products

Basic qPCR Protocol

The following basic protocol serves as a general guideline and a starting point for any qPCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of qPCR Hotstart Taq DNA Polymerase, primers, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube:

1.1 Recommended qPCR assay with SYBR Green I dye

Component	Amount	Final concentration
10× qPCR Buffer（Mg^{2+} Plus）	5 μl	1×
dNTPs (10 mM each)	1 μl	0.2 mM
F primer (10 μM)	2 μl	0.4 μM
R primer (10 μM)	2 μl	0.4 μM
20× SYBR Green I	0.5-2.5 μl	0.2-1×
qPCR Hotstart Taq DNA Polymerase（5 U/μl）	0.5-1 μl	2.5-5 U
template DNA	1-4 μl, <1 μg	<1 μg
ddH_2O	To 50 μl	-
ROX reference dye*	Variable	-

1.2 Recommended qPCR assay with probe

Component	Amount	Final concentration
10× qPCR Buffer（Mg^{2+} Plus）	5 μl	1×
dNTPs (10 mM each)	1 μl	0.2 mM
F primer (10 μM)	2 μl	0.4 μM
R primer (10 μM)	2 μl	0.4 μM
Probe (10 μM)	0.5-2.5 μl	0.1-0.5 μM
qPCR Hotstart Taq DNA Polymerase（5 U/μl）	0.5-1 μl	2.5-5 U
template DNA	1-4 μl, <1 μg	<1 μg
ddH_2O	To 50 μl	-
ROX reference dye*	Variable	-

*For some types of instruments, ROX is required to determine Ct values accurately. ROX dosage should refer to the specific instrument description. The following table is for reference only:

Table of Instrument Guide

Instrument	Conc. of ROX (100×)
ABI® PRISM® 7000, 7700, 7900HT, ABI® 7300 qPCR Systems, GeneAmp® 5700, StepOne™, and the StepOnePlus™	1-2% ROX
ABI® 7500 qPCR Systems, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™ Mx3005P™ and Mx4000™	0.2% ROX
BioRad iCycler MiniOpticon, Opticon 2, Chromo 4, iQ5; Roche LightCycler 480; MJ Research DNA Engine Opticon 2, Chromo 4; Corbett Rotogene 3000, 6000	No ROX

2. Setup the plate

Transfer the reaction mixture to PCR tubes/plates. Reaction volumes can be reduced to 10 μl if the instrument supports a low volume system.

Cap or seal the reaction tubes/plates then centrifuge briefly to spin down the contents and eliminate any air bubbles.

3. Preform qPCR using the following thermal cycling condition

Set the thermal cycling conditions using default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters of the specific primers.

Standard 3-step PCR mode:

Initial denaturation	95°C	3 min	Holding Stage
Denaturation	95°C	10 sec	Cycling Stage 40 Cycles
Annealing [a]	60°C	15 sec	
Extension [b]	72ºC	20 sec	

a The instrument do signal acquisition at this stage when doing probe-based qPCR. The usual annealing temperature is 55-65°C. The annealing temperature is generally set to Tm-5 ºC of the primer used, generally not less than 55 ºC. (melting temperature, Tm). Set the annealing time according to the instrument guide.

b The instrument do signal acquisition at this stage when doing dye-based qPCR.

2-step PCR mode:

Initial Denaturation	95°C	3 min	Holding Stage
Denaturation[a]	95°C	10 sec	Cycling Stage 40 Cycles
Annealing & Extension*	60°C	30 sec	

* The instrument do signal acquisition at this stage. Set the annealing time according to the instrument guide.

If the reaction performs not well, it is recommended to adopt a 3-step amplification procedure.

4. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Important Notes

Assay Design

We recommend using previously validated assays or using dedicated qPCR design software such as Beacon Designer 7 when designing Probe-based assays (www.PremierBiosoft.com).

Lyophilized primers and probes should be resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. DNA kept frozen in a nuclease-free environment should be stable for years. We find it convenient to initially prepare a 100 µM freezer stock (which should be thawed relatively infrequently).

Optimal primer concentration should be determined empirically. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the qPCR reaction. The optimal primer concentration range is 100-400 nM.

Optimal probe concentration should be determined empirically. The optimal probe concentration range has generally been found to be 0.1-0.5 µM.

Template

Genomic DNA, plasmid DNA, or cDNA can be used as template. For optimal quantitative results use up to 20ng of genomic DNA or plasmid DNA per 20 µl reaction (for smaller volumes, the amount of template should be decreased equivalently). Using greater amounts of template may reduce the maximum fluorescence signal due to binding of the probe to the template. For two-step RT-PCR, use either undiluted or diluted cDNA generated from up to 1µg of total RNA. The volume of the cDNA added (from the RT reaction) should not exceed 10% of the final PCR volume (e.g., for a 20 µl qPCR reaction, use up to 2.0 µl of undiluted cDNA).

Primers

Careful primer design and purification (HPLC-purified primers are recommended) is particularly important in order to minimize loss in sensitivity due to the production of nonspecific amplification products

in qPCR. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the PCR reaction (0.1-1.0 μM of each primer). For optimal results, design primers that amplify PCR products 50-150 bp in length. The primers should exhibit a melting temperature (Tm) of approximately 60ºC. We recommend designing primers specifically for amplification of cDNA derived from mRNA. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

Magnesium chloride

The concentration of $MgCl_2$ affects the binding dynamics of primers and probes to template DNA. The higher the final $MgCl_2$ concentration in the PCR reaction, the greater the binding affinity of the primers and probe for target DNA. 2× qPCR Buffer provides $MgCl_2$ at a final concentration which is suitable for most targets.

Guidelines for preventing contamination of qPCR reaction

During qPCR 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 qPCR mixture, perform thermal cycling and analyze PCR products in separate areas.

• Set up a 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, exodeoxyribonucl- eases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.