PRODUCT INSERT

PUREGENE GENETIX BRAND

G-Script One Step Probe RT-PCR Supermix

Store at -20°C

Cat. No.	Pack Size
PGK160-A	100 Rxns

ORDERING INFORMATION

Component	PGK160-A
G-Script One Step Probe RT-PCR Supermix, 4X	500 µL
Nuclease Free Water (NFW)	1000 µL

DESCRIPTION

Puregene G-Script One Step Probe RT-PCR Supermix combines antibody-mediated hot-start polymerase, reverse transcriptase, RNase Inhibitors, dNTPs and $MgCl_2$ in one tube with the latest advances in buffer chemistry and PCR enhancers.

G-Script One step Probe RT-PCR Supermix accurately amplifies target DNA and RNA under fast thermal cycling conditions. It gives excellent results in multiplex assays even in the presence of difficult inhibitors found in sputum and stool samples.

FEATURES

- G-Script One step Probe RT-PCR Supermix is a single tube, ready to use Supermix.
- Detection chemistry: Probes
- Multiplex capability: Upto 5 targets
- Inhibitor Tolerance: Tested with ethanol and hematin. Compatible with upto 10 µL of crude lysates
- Hot start: Yes; Activation time: 10 min
- Sample input: 1 pg - 1 µg
- Sensitivity: 10 copies in a 5-plex reaction
- Reference dye: Tented universal dye preblended in the Supermix
- Shelf life: -20 degrees, 12 months; 4 degrees after thawing 1 week

PROTOCOL

1. Gently vortex and briefly centrifuge G-Script One step Probe RT-PCR Supermix (4X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 20 µL reaction:

G-Script One step Probe RT-PCR Mix, 4x	5 µL
Primer-Probe Mix, 20x	1 µL
Template* (1 pg – 1 µg)	x µL
Water	Upto 20 µL
Total volume	20 µL

* RNA and DNA template from extracted or crude sample lysate. Template volume optimization required.

3. Gently vortex the samples and spin down.
4. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temp.°C	Time	No. of cycles
Reverse transcription**	50	10 min	1
Polymerase activation	95	2 min	1
Denaturation	95	5 sec	45
Annealing/Extension**	60	20 sec	45

**When multiplexing, the reverse transcription reaction time can be extended up to 20 minutes and/or the temperature can be increased up to 55° C and the annealing/ extension time can be extended up to 60 seconds and/or the temperature can be increased up to 65° C.

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 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.

GUIDELINES FOR PRIMER DESIGN

Follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:
Tm= 4 (G + C) + 2 (A + T),
where G, C, A, T represent the number of respective nucleotides in the primer.
If the primer contains more than 25 nucleotides we recommend using specialized computer to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.
All routine DNA purification methods can be used to prepare the template. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 M. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products. For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for Taq DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72°C for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb. For amplification of longer templates (>6 kb) a reduction of the extension temperature to 68°C is required to avoid enzyme inactivation during prolonged extension times.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning the final extension step can be omitted.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25 µL of DreamTaq PCR Master Mix (2X) with 1 µg of pUC19 DNA in 50 µL of reaction mixture for 4 hours at 37°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 25 µL of GeneTaq PCR Master Mix (2X) with 1 µg of digested DNA in 50 µL of reaction mixture for 4 hours at 37°C.

Ribonuclease Assay

Less than 0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µL GeneTaq PCR Master Mix (2X) with 1 µg of [3H]-RNA in 50 L of reaction mixture for 4 hours at 37°C.

Functional Assay

GeneTaq PCR Master Mix (2X) was tested for amplification of 20 kb DNA fragment.