

GeneFast Universal SYBR Green Master Mix (2X)

Store at -20°C

Cat. No.	Concentration	Pack Size
PGK030A	2X	500 rxn x 20ul

Shipping Conditions: On Dry Ice or Blue Ice

Storage and Stability: Store at -20°C and for longer use store at -70°C. Excessive freeze/thawing is not recommended. Since SYBR Green I is light sensitive, it is important to avoid prolonged exposure to light. When stored under optimum conditions, the reagents are stable for a minimum of 6 months from date of purchase.

Quality Control: The GeneFast Universal SYBR Green Master Mix and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Safety Precautions: Harmful if swallowed. Irritating to eyes, respiratory system and skin.

COMPONENT

PGK030A	Vol.
GeneFast Universal SYBR Green Master Mix (2X)	5mL
Water, nuclease-free	5 x 1mL

DESCRIPTION

GeneFast Universal SYBR Green Master Mix is a premixed universal 2X master mix for real-time PCR workflows. It is ready to use solution containing SYBR Green dye, Dual-Lock Taq DNA Polymerase, dNTPs with dUTP/dTTP blend, heat-labile UDG, ROX passive reference dye, and optimized buffer components.

It is formulated to amplify targets for accurate expression analysis of gene when combined with the user-supplied primer sets and template. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up.

FEATURES AND BENEFITS:

Simple and reproducible: A choice of optimized kits for different platforms, just add primers and template, reducing possible errors in set-up. It provides reproducibility in CTs over a wide dynamic range for a variety of targets tested.

Specific and sensitive: For detection of a wide range of template concentration, even from hard to obtain and low copy number samples. GeneFast Universal SYBR Green Master Mix uses the Dual-Lock Taq DNA polymerase enzyme that combines two unique hot-start mechanisms to help control its activity and to prevent undesirable early activity of the polymerase at low temperatures.

Contamination control: Uracil-DNA glycosylase (UDG) and dUTP are added to the GeneFast Universal SYBR Green Master Mix which enables the degradation of any previously amplified PCR products. This helps in preventing contamination of subsequent qPCR reactions.

Fast protocols: Can be used with the new generation of fast cyclers without compromising performance

Instrument Compatibility

Specifically suitable for use on real-time instruments that measure ROX signal like ABI 7000, 7300, 7700, 7900, 7900HT and StepOne™. However, the kit is also compatible with several instruments that do not require the use of ROX™, such as the Qiagen Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler 480.

PROTOCOL

1. Gently vortex and briefly centrifuge GeneFast Universal SYBR Green Master Mix (2X) after thawing.

2. Place a thin-walled PCR tube on ice and add the following components for each 20 µL reaction. The volumes given below are based on a standard 20µl final reaction mix and can be scaled accordingly

GeneFast Universal SYBR Green Master Mix (2X)	10µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	1 ng-10 ng cDNA
	10-100 ng gDNA
Water, nuclease-free	upto 20 µL
Total volume	20 µL

3. Gently vortex the samples and spin down.

4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.



PRODUCT INSERT



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5. Perform PCR using the recommended real time thermal cycling conditions outlined below:

Fast cycling mode (primer Tm ≥60°C)

Step	Temp. °C	Time	No. of Cycles
UDG Activation	50	2 min	1
Activation	95	2 min	1
Denature	95	1-3 s	40
Annealing/Extension	60	30 s	

Standard cycling mode (primer Tm ≥60°C)

Step	Temp. °C	Time	No. of Cycles
UDG Activation	50	2 min	1
Activation	95	2 min	1
Denature	95	15 s	40
Annealing/Extension	60	1 min	

Standard cycling mode (primer Tm <60°C)

Step	Temp. °C	Time	No. of Cycles
UDG Activation	50	2 min	1
Activation	95	2 min	1
Denature	95	15 s	40
Annealing	55-60	15 sec	
Extension	72	1 min	

6. Set the PCR to perform a default dissociation step which can be run for 72 hours after the real time PCR completion if the plate is stored in dark. If the plate is exposed to light, then the dissociation step can be performed upto 24 hours after the real time PCR run.

Conditions for Dissociation curve (melt curve)

Step	Ramp rate	Temp °C	Time
1	1.6°C/sec	95	15 s
2	1.6°C/sec	60	1 min
3	0.15°C/sec	95	15 s

7. Start the run

Result Analysis

For analyzing the results, first check the amplification plots. Then calculate the baseline and Threshold cycles (Ct) using the instrument software. By using dissociation curves, check for non-specific amplification. Perform relative or absolute quantitation.

GENERAL CONSIDERATIONS

To help prevent any carry-over DNA contamination we recommend that separate areas be maintained for PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in the PCR set-up area.

Primers: The sequence and concentration of primer as well as the amplicon length can be critical for specific amplification, yield and overall efficiency of any real-time PCR. We strongly recommend taking the following into consideration when designing and running your PCR reaction:

Use primer-design software, such as Primer3 or visual OMPTM (<http://frodo.wi.mit.edu/primer3/> and DNA Software, Inc; <http://dnasoftware.com/> respectively). Primers should have a melting temperature (Tm) of approximately 60°C. Optimal amplicon length should be 50-150bp.

A final primer concentration of 250nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1µM.

Use equimolar primer concentrations.

When amplifying from cDNA use gene-specific primers. If possible use intron-spanning primers to avoid amplification from genomic DNA.

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. Also, the template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following should be considered when using genomic DNA and cDNA templates:

Genomic DNA: use up to 1µg of complex (e.g. Eukaryotic) genomic DNA in a single PCR. We recommend using the Nucleopore Genomic DNAMini Kit for high yield and purity from both prokaryotic and eukaryotic sources.

cDNA: the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene. We suggest using 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-[®] TM step RT-PCR, we recommend using the Puregene GENESCRIP[®] First Strand cDNA Synthesis Kit for reverse transcription of the purified RNA. For [®] high yield and purity of RNA, use RNASure Mini Kit (Nucleopore).

MgCl₂: The MgCl₂ concentration in the 1x reaction mix is 3mM. In the majority of qPCR conditions this is optimal for both the reverse transcriptase and the hot-start DNAPolymerase. If necessary, we suggest titrating the MgCl₂ to a maximum of 5 mM.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no template control (NTC), replacing the template with PCR-grade water. When performing a two-step RTPCR, set-up a no RT control as the NTC for the PCR.



GENETIX BRAND

Tel: +91-11-4502-7000 Email: info@genetixbiotech.com Website: www.genetixbiotech.com