Protocol
HB Probe Based qPCR Master Mix

Catalog Number	Size	Concentration
HBP01-0100	100 reactions (20 µl vol)	2X

Storage Conditions

Stable for up to 3 months when stored at 4°C, and for up to 24 months when stored at -20°C.

Description

HB Probe Based qPCR Master Mix is a specialized and convenient solution for probe-based real-time PCR. It is a ready-to-use master mix that is concentrated at a 2x level for optimal performance. This master mix is specifically optimized for compatibility with a wide range of commercially available real-time PCR systems, including both ROX-independent and ROX-dependent systems. HB Probe Based qPCR Master Mix contains hot-start Taq DNA polymerase, dNTPs, MgCl2, enhancers, stabilizers, and all the essential components necessary for a successful PCR reaction. It provides a reliable and efficient amplification of your target sequences in a probe-based real-time PCR setup.

Kit Content(s)

2X Universal qPCR Master Mix

Required materials but not provided

- A compatible real-time PCR instrument
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

Instrument Compatibility

The provided Master Mix is designed to be compatible with the majority of real-time PCR systems available on the market.

Reaction Setup

1. To thaw HB Probe Based qPCR Master Mix and other frozen reaction components, bring them to a temperature of 4°C. Make sure to combine the solutions thoroughly and briefly centrifuge to ensure complete collection. Store the mixture at 4°C, away from light.
2. Prepare enough assay Master Mix for all reactions by adding all the necessary components, excluding the DNA template. Follow the recommendations provided in Table 1 (shown below) when preparing the assay Master Mix. For best results, prepare the Master Mix on ice or at room temperature.

Table 1. Reaction Setup			
Components	**Volume per 20 µl Reaction**	**Volume per 10 µl Reaction**	**Final Concentration**
HB Probe Based qPCR Master Mix (2x)	10 µl	5 µl	1x
Forward and reverse primers	Variable	Variable	300–500 nM each primer
Fluorogenic probe(s)	Variable	Variable	150–250 nM each
DNA template **(add at step 4)**	Variable	Variable	cDNA: 1pg–10ng Genomic DNA: 50ng-250ng
Nuclease-free H_2O	Variable	Variable	——
Total reaction mix volume	20 µl	10 µl	——

3. Thoroughly combine the assay Master Mix to ensure consistency. Dispense the solution equally into each qPCR tube or the wells of a qPCR plate. Use good pipetting practice to maintain assay precision and accuracy.
4. Add DNA samples to the PCR tubes or wells containing the assay Master Mix from Table 1. If necessary, also add DNase-free H2O. Seal the tubes or wells with flat caps or optically transparent film. For thorough mixing of reaction components, vortex the mixture for approximately 30 seconds or more.
5. Spin the tubes or plate to eliminate any air bubbles and collect the reaction mixture at the bottom of the vessel.
6. Set up the thermal cycling protocol on your real-time PCR instrument according to Table 2. Please note that optimization may be required for optimal performance.
7. Load the PCR tubes or plate into the real-time PCR instrument and start the run.
8. Perform data analysis following the specific instructions provided for your instrument.

* Set up the thermal cycler to run for 35-45 cycles according to the following parameters:

Table 2. Thermal Cycling Protocol	
Initial Denaturation	3-5 minutes at 95°C (5 mins for GC rich or complex templates)
Denaturation	15 seconds at 95°C
Annealing & Extension	60 seconds at 60°C and Plate Read

Note: The optimal conditions for amplification may vary depending on the primers and thermal cycler being used. It may be necessary to optimize the system based on individual primers, template, and thermal cycler specifications.

Template

Ensure that you have purified high-quality DNA for a successful PCR reaction. Please refer to Table 1 for the recommended final concentration of DNA template.

Important notes

- Before use, gently shake the components to avoid foaming.
- Use low-speed centrifugation when necessary.
- Always wear a lab coat, disposable gloves, and other appropriate protective equipment during the procedure.

Troubleshooting

If you encounter any issues during the quantification of nucleic acid targets using the kit, please refer to Table 3 for troubleshooting guidance.

Table 3. Troubleshooting

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.

Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer-dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

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