Probe Multiplex Real-Time PCR Master Mix (2X)

Product Manual

Version 1.0

This product is intended for research use only. Do not use this product for diagnostic purposes.

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Intended use

This product is intended for real-time qPCR applications using the fluorescent probe chemistry for research purposes only. Please do not use this product in diagnostic applications.

This master mix is compatible with multiplex qPCR assays such as gene expression assays, genotyping assays, and pathogen detection assays.

We have optimized this product to be compatible with all commercially available real-time PCR machines.

Principle

Real-time qPCR is modification of the traditional PCR technique, in which amplification of DNA or cDNA (which is prepared from an RNA template) is monitored in real time by fluorescence.

Traditionally, the PCR products are run on a gel then visualized under a transilluminator. This type of analysis is termed end-point analysis because it only reflects the final reaction products.

On the other hand, incorporation of a specific fluorescent probe in the reaction mix allows to monitor the reaction progression after each cycle. The fluorescent probe is quenched by incorporating a quencher molecule, usually at the 3' end of the probe, and binds to the target DNA/cDNA sequence upstream of the forward prime (or alternatively the reverse primer). Upon extension of the forward primer, the DNA polymerase cleaves the fluorescent probe in a 5'–3' exonuclease reaction, thereby dissociating the quencher molecule from the probe, and thus emitting a fluorescent signal. Therefore, after each replication, or doubling, of the targeted DNA/cDNA, the emitted fluorescence is also doubled. Fluorescence is then measured after each cycle by the real-time PCR machine, and information about the starting template can be inferred from fluorescence data.

This product contains all components needed for an efficient real-time qPCR reaction: a robust DNA polymerase, pure dNTPs, stabilizers, and enhancers. The user only needs to add the template and our optimized assays (or their own custom assays).

Kit contents and storage conditions

This kit contains 5 tubes of 1 mL Probe Multiplex Real-Time PCR Master Mix. Store at -20°C immediately upon receipt.

Running qPCR reactions

1. Let the Probe Multiplex Real-Time PCR Master Mix, Haven's optimized custom assays (or the user designed primers and probes), and templates thaw completely at room temperature then put them on ice; for optimal results, work under minimal lighting and finish setting the experiment as quickly as possible

2. Determine the number of reactions needed for each specific assay

   Example: if you wish to run duplicate reactions for 10 different samples, you will perform:

   $$2_{\text{replicates}} \; X \; 10_{\text{samples}} = 20_{\text{qPCR reactions}}$$

3. Prepare an assay master mix sufficient for the total number of reactions (determined in step2) plus 10% to account for pipetting errors; this is achieved by mixing the following components for final reaction volumes of 20 µL (*do not add the DNA/cDNA samples yet*):

Component	Volume per reaction	***Example:*** ***Volume per 20+2 reactions***	Final concentration
Probe Multiplex PCR MM	10 µL	*220 µL*	1 X
Haven's custom assay (20X)	1 µL	*22 µL*	1 X
Template (DNA or cDNA)	Variable	*Variable*	10 pg – 100 ng (cDNA) 1-10 ng (DNA)
PCR-grade water	Up to 20 µL	*Up to 440 µL*	–

If user designed primers and probes are used, refer to the following table:

Component	Volume per reaction	***Example:*** *Volume per 30+3 reactions*	Final concentration
Probe Multiplex PCR MM	10 µL	*330 µL*	1 X
Forward primer	Variable	*Variable*	200 – 800 nM
Reverse primer	Variable	*Variable*	200 – 800 nM
Probe	Variable	*Variable*	100 – 500 nM
Template (DNA or cDNA)	Variable	*Variable*	10 pg – 100 ng (cDNA) 1-10 ng (DNA)
PCR-grade water	Up to 20 µL	*Up to 660 µL*	–

4. If you are testing more than one assay, prepare an assay master mix for each assay as in step3

5. Load the appropriate amount of the assay master mix into the allocated PCR plate wells or PCR tubes

   Example: if the amount of DNA/cDNA template was determined in step3 to be 2 µL, load 18 µL of the assay master mix into each allocated well or PCR tube

6. Add the appropriate amount of DNA/cDNA template, as determined in step3, to each allocated well

7. Program the real-time thermal cycler as follows:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 min	Hold
Denaturation	95°C	15 sec	40-45 cycles
Annealing/extension*	57-65°C**	60 sec	

* data collection in this step
** depending on the assay

8. Analyze data using your real-time thermal cycler's software

Quality control

Each lot is tested for certain preset parameters to ensure compliance with manufacturing procedures.

Support

For questions, suggestions, or technical support, feel free to email our technical team at support@havensci.com.