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

Product Manual

Version 2.0

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

Table of Contents

Intended use 3

Principle 3

Kit contents and storage conditions 4

Required Materials That Are Not Included in the Kit 4

Compatible Instruments 5

Good PCR Practices 6

Running qPCR reactions 7

Quality control 9

Support 9

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 comes in two sizes:

- PCR6905: contains 5 tubes of 1 mL Probe Multiplex Real-Time PCR Master Mix and 200µL of ROX reference dye.
- PCR6901: contains 1 tubes of 1 mL Probe Multiplex Real-Time PCR Master Mix and 200µL of ROX reference dye.

Store at -20°C immediately upon receipt.

Required Materials That Are Not Included in the Kit

In addition to the kit, you will need the following materials:

- Haven's customized gene expression assays *OR* your own PCR primers
    - Please contact our sales team at sales@havensci.com for ordering the customized gene expression assays
- DNA or cDNA samples
- PCR thermal cycler
- One of the following:
    - PCR plates compatible with your PCR thermal cycler, an adhesive seal and an applicator to seal off the PCR plate, and a PCR plate microcentrifuge, OR
    - PCR strips and strip caps compatible with your PCR thermal cycler, OR
    - PCR tubes compatible with your PCR thermal cycler
- Pipettes and filtered pipette tips in the range of 1–1,000µL
- 1.5mL or 2.0mL RNase-free microcentrifuge tubes
- Microcentrifuge with 1.5mL/2.0mL rotor
- (highly recommended) Ice or a cooled PCR stand/rack
- (optional) RNase-free water

Compatible Instruments

The Probe Multiplex Real-Time PCR Master Mix is compatible with the following real-time PCR systems:

- 7300 Real-Time PCR System (ABI, 4351101)
- 7500 Real-Time PCR System (ABI, 4351104)
- StepOne Real-Time PCR System (ABI, 4376357)
- StepOnePlus Real-Time PCR System (ABI, 4376600)
- QuantStudio 3 Real-Time PCR System (ABI, A28567)
- QuantStudio 5 Real-Time PCR System (ABI, A28574)
- QuantStudio 7 Flex Real-Time PCR System (ABI, 4485688)
- CFX Connect Real-Time PCR Detection System (Bio-Rad, 1855201)
- CFX Duet Real-Time PCR System (Bio-Rad, 12016265)
- CFX96 Real-Time PCR Detection System (Bio-Rad, 185-5096)
- CFX Opus 96 Real-Time PCR System (Bio-Rad, 12011319)
- Rotor-Gene Q 5plex Platform (Qiagen, 9001570)
- LightCycler® 480 Instrument (Riche Diagnostics, 05015278001)
- AriaMx Real-time PCR System (Agilent, G8830A)

Please consult us about the compatibility of your thermal cycler by sending an email to support@havensci.com.

Good PCR Practices

Contamination is the worst enemy of PCR. Here, we list a few measures that could help you avoid contamination:

- Have physically separate workstation for PCR preparation, sample loading, and nucleic acid extraction. Each workstation should have its own dedicated pipettes and other small instruments, and should preferably be in a separate room.
- Always work under a cabinet (laminar flow for PCR preparation, and safety cabinet for nucleic acid extraction and sample loading).
- Use filtered tips. They are more expensive, but the small extra cost may save you valuable time, effort, and money down the line.
- If you need to analyze the PCR product after PCR cycling has finished, never unseal the plate near your PCR preparation or sample loading workstations. Even the smallest of aerosols could contain tens of millions of PCR templates.

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 20+2 reactions***	Final concentration
Probe Multiplex PCR MM	10 µL	*220 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 440 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.