EverGreen Universal qPCR Master Mix (2X)

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

Made in SAUDI ARABIA
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Intended Use

This product is intended for real-time qPCR applications using the intercalated dye chemistry. It is compatible with unmodified oligos (primers) only, so the use of dye-labelled oligos is not possible. Only DNA or cDNA samples can be used with this master mix. For RNA samples please refer to our One-Step EverGreen RT-qPCR Master Mix (Cat. No. PCR5905).

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Principle

Real-time qPCR is a 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 type of intercalating dyes in the reaction mix allows to monitor the reaction progression after each cycle. These intercalating dyes do not participate in the reaction, but bind strongly to double-stranded DNA. Upon binding, their fluorescence increases approximately 1,000-fold. Thus, after each replication, or doubling, of the targeted DNA element, the emitted fluorescence due to binding is also doubled. Fluorescence is then measured after each cycle by the real-time PCR machine, and the amount of the starting template can be inferred from fluorescence data.

This kit contains all components needed for an efficient real-time qPCR reaction: a robust DNA polymerase, pure dNTPs, stabilizers, enhancers, and the intercalating dye. The user only needs to add the

Kit Contents and Storage Conditions

his kit contains 5 tubes of 1 mL EverGreen Universal qPCR Master Mix. Store at -20°C immediately upon receipt. Minimize exposure to light

Required Materials That Are Not Included in the Kit

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

1. **Haven's customized gene expression assays OR your own PCR primers**
   a. **Please contact our sales team at sales@havensci.com for ordering the customized gene expression assays**
2. **DNA or cDNA samples**
3. **Real-time PCR thermal cycler (see next section: Compatible Instruments)**
4. **PCR plates compatible with your real-time PCR thermal cycler**
5. **Optical adhesive seal and an applicator to seal off the PCR plate**
6. **PCR plate microcentrifuge**
7. **Pipettes and filtered pipette tips in the range of 1–1,000µL**
8. **1.5mL or 2.0mL RNase-free microcentrifuge tubes**
9. **Microcentrifuge with 1.5mL/2.0mL rotor**
10. **(highly recommended) Ice or a cooled PCR stand/rack**
11. **(optional) RNase-free water**

Compatible Instruments

The EverGreen Universal qPCR Master Mix kit is compatible with the following real-time PCR systems:

1. 7300 Real-Time PCR System (ABI, 4351101)
2. 7500 Real-Time PCR System (ABI, 4351104)
3. StepOne Real-Time PCR System (ABI, 4376357)
4. StepOnePlus Real-Time PCR System (ABI, 4376600)
5. QuantStudio 3 Real-Time PCR System (ABI, A28567)
6. QuantStudio 5 Real-Time PCR System (ABI, A28574)
7. QuantStudio 7 Flex Real-Time PCR System (ABI, 4485688)
8. CFX Connect Real-Time PCR Detection System (Bio-Rad, 1855201)
9. CFX Duet Real-Time PCR System (Bio-Rad, 12016265)
10. CFX96 Real-Time PCR Detection System (Bio-Rad, 185-5096)
11. CFX Opus 96 Real-Time PCR System (Bio-Rad, 12011319)
12. Rotor-Gene Q 5plex Platform (Qiagen, 9001570)
13. LightCycler® 480 Instrument (Riche Diagnostics, 05015278001)
14. 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 EverGreen Master Mix, Haven's customized gene expression assays (or your own primers) and DNA/cDNA samples 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 triplicate reactions for 10 different samples, you will perform:

3. $3_{\text{replicates}} \times 10_{\text{samples}} = 30_{\text{qPCR reactions}}$

Prepare an assay master mix sufficient for the total number of reactions (determined in step2) plus 5% 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 30 reactions + 5%***	Final concentration
EverGreen qPCR MM	10 µL	*315 µL*	1 X
Haven's custom assay (20X)	1 µL	*31.5 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 630 µL*	–

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

Component	Volume per reaction	***Example:*** ***Volume per 30 reactions + 5%***	Final concentration
EverGreen qPCR MM	10 µL	*315 µL*	1 X
Forward primer	Variable	*Variable*	200 – 800 nM
Reverse primer	Variable	*Variable*	200 – 800 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 630 µL*	–

4. If you are testing more than one assay, prepare a 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
Reverse transcription	45-55°C*	10-15 min	Hold
Initial denaturation	95°C	3 min	Hold
Denaturation	95°C	15 sec	40 cycles
Annealing/extension**	57-65°C*	60 sec	
Melting curve	65 to 95°C	0.2°C/sec ramp up rate	Hold

* data collection in this step
* depending on the assay

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

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Support

Support

For questions, suggestions, or technical support, feel free to contact us
by email on: support@havensci.com

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Quality Control

Quality Control

Each lot is tested against predetermined parameters to ensure consistent
performance between all lots.

HavenSci

Evergreen qPCR Master Mix (2X)
Product manual (PCR5505)
Version 3.0
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