SteadyTaq PCR Master Mix, HotStart (2X)

Product Manual

Version 3.0

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 traditional PCR applications for PCR beginners as well as experienced users. Both DNA and cDNA are compatible with this master mix. For RNA samples, please reverse transcribe them to cDNA first by using our RT Ace First Strand cDNA Synthesis Kit (Cat. No. PCR3005, PCR3105).

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Principle

PCR works by successive replication of a specific DNA element by a DNA polymerase enzyme. Each replication process is termed a "PCR cycle", or simply a cycle. The cycle starts when the DNA sample is denatured at high temperatures, usually 95°C. Afterwards, the temperature is lowered so that synthetic oligo nucleotides, termed "primers", anneal to specific regions on the DNA template. These primers are added to the reaction to guide the DNA polymerase to a specific DNA element that is targeted for PCR. Simply put, primers show the DNA polymerase where to work. Once annealed, the DNA polymerase extends these primers along the template DNA by incorporation of nucleotides to form a double stranded DNA. The newly generated dsDNA is denatured by increasing the temperature, and another cycle ensues. This successive doubling of the template can theoretically generate over 33 million PCR products for each 1 molecule of the starting template after 25 cycles, as long as reactants are in excess. It is important to note that, in the vast majority of applications, these PCR products are copied from a specific targeted region of the template, not from the whole template.

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

Our SteadyTaq PCR master mix contains our highly dependable, antibody-mediated HotStart SteadyTaq DNA polymerase enzyme, dNTPs, and a proprietary buffer that enhances specificity to the targeted sample. All you need to add is the sample and primers. We also supply our SteadyTaq enzyme alone with a standard buffer for experienced users who wish to formulate their own buffers. Please refer to our SteadyTaq DNA Polymerase kits (Cat. No. PCR1105-250, PCR1105-500, PCR1105-1000).

Kit contents and storage conditions

This kit comes in two sizes:

- PCR9505: contains 5 tubes of 1 mL SteadyTaq PCR Master Mix, HotStart.
- PCR9501: contains 1 tubes of 1 mL SteadyTaq PCR Master Mix, HotStart.

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

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.
- When analyzing the PCR product by electrophoresis, never unseal the plate or open PCR tubes near your PCR preparation or sample loading workstations. Even the smallest of aerosols could contain tens of millions of PCR templates.

Running PCR reactions

1. Let the SteadyTaq 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, 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:

   $$3_{\text{replicates}} \; X \; 10_{\text{samples}} = 30_{\text{PCR reactions}}$$

3. 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
SteadyTaq PCR MM	10 µL	*315 µL*	1 X
Haven's custom assay (20X)	1 µL	*31.5 µL*	1 X
DNA Template	Variable	*Variable*	1–1,000 ng
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
SteadyTaq PCR MM	10 µL	*315 µL*	1 X
Forward primer	Variable	*Variable*	200 – 800 nM
Reverse primer	Variable	*Variable*	200 – 800 nM
DNA Template	Variable	*Variable*	1–1,000 ng
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 thermal cycler as follows:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	3 min	Hold
Denaturation	95°C	15 sec	30-35 cycles
Annealing	55-65°C*	15 sec	
Extension	72°C	1 min per 1kb of PCR product	
Final extension	72°C	5 min	Hold

* depending on the assay

8. Run 5µL of the PCR product on gel electrophoresis according to your internal protocols to visualize your PCR product; if the band is too bright, dilute the PCR product in a 5X factor

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.