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2×HotStart *Taq* PCR Master Mix with Dye

Catalog Number: D015-1, D015-2

Table 1. Contents and Storage

Content	D015-1	D015-2	Storage	Stability
2×HotStart *Taq* PCR Master Mix	5×1 mL	10×1 mL	-20 ºC, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.

Product Description

ABP HotStart *Taq* PCR Master Mix is a 2× concentrated solution of HotStart *Taq* DNA Polymerase, dNTPs, and all of the components required for PCR, except DNA template and primers. HotStart *Taq* PCR Master Mix provides robust and reliable performance in PCR amplification. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. HotStart *Taq* PCR Master Mix contains inert, non-toxic dyes to visualize PCR mixing step, and also allows direct loading of PCR products on to gels for electrophoresis.

The HotStart *Taq* DNA Polymerase is a recombinant Taq DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures, whose enzyme activities can be re-activated after 1-3 minutes of incubation at 95°C. The HotStart *Taq* DNA Polymerase uses amplification conditions for regular *Taq* DNA Polymerase, except no polymerase activity will be present before the onset of thermal cycling. This prevents nonspecific DNA amplification and primer dimer formation.

Special Features

- Convenient, ready-to-use mix.
- Hot start.
- Generates PCR products with 3'-dA overhangs.
- Incorporates modified nucleotides (e.g., biotin-, digoxigenin-, fluorescently-labeled nucleotides).

Applications

- Routine PCR.
- High sensitivity PCR
- High throughput PCR.
- Hot Start PCR.

Composition of HotStart *Taq* PCR Master Mix (2X)

0.05 U/μL HotStart *Taq* DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP).

General Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of HotStart *Taq* DNA polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized. Critical parameters and troubleshooting information are documented in reference 1.

Assemble PCR reactions in a nuclease-free environment. Use of “clean” dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare the following reaction mixture in a PCR tube on ice:

Component	Volume	Final Concentration
Template DNA	x µL	1-500 ng
2×HotStart *Taq* PCR Master Mix	25 µL	1×
Forward Primer (10 µM)	1.5 µL	300 nM
Reverse Primer (10 µM)	1.5 µL	300 nM
Nuclease-free H_2O	to 50 µL	-

3. Mix, and then briefly centrifuge the contents.
4. Perform PCR using the recommended thermal cycling conditions outlined below:

Steps	Temperature	Duration	Cycle
Initial Denaturation	95°C	1-3 min	1
Denaturation	95°C	10 sec	25-35
Annealing*	56-64°C	10 sec	
Extension	72°C	15-30 sec/kb	
Final Extension	72°C	10 min	1
Holding	4°C	-	1

5. Analyze the amplification products by agarose gel electrophoresis.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

• Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.

• Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.

• Wear fresh gloves for DNA purification and reaction set up.

• Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.

• Always perform “no template control” (NTC) reactions to check for contamination.

Reference

Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, eds. (1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA.