# ama*R* OnePCR™ HotStar

Cat. No.: SM216-0250 Size: 250 Reactions (2 × 1.25 ml)
Cat. No.: SM216-0005 Size: 5 Reactions (1 × 50 µl)

## Description

Ama*R* OnePCR™ HotStar is a ready-to-use PCR reaction mixture. Simply add primers, template, and water, the reagent will execute primer extensions and other molecular biology applications. Ama*R* OnePCR™ HotStar is a pre-mixed solution containing hot-start *Taq* DNA polymerase, PCR reation buffers, dNTPs, gel loading dyes, enhancer, and fluorescence dye. Ama*R* OnePCR™ HotStar contains hot-start *Taq* DNA polymerase, which exhibits non-template–dependent terminal transferase activity that adds a 3' deoxyadenosine to product ends, and 5'→3' exonuclease activity (but not 3'→5' exonuclease activity). The hot-start *Taq* DNA polymerase is *Taq* DNA polymerase complexes with a proprietary antibody that blocks activity at room temperatures. When heat to 95°C, the enzyme is restored, providing an automatic "hot start" for *Taq* DNA polymerase in PCR, increasing the sensitivity and specificity of PCR reaction. Ama*R* OnePCR™ HotStar contains a red tracking dye, provide a safe, non-toxic and non-mutagenic alternative to Ethidium Bromide for instantaneous band visualization, the dye is environmentally friendly containing no hazardous chemicals. The Ama*R* OnePCR™ HotStar contains only a single fast-running tracking dye that migrates at approximately 10 base pair in a 1% agarose gel. Ama*R* OnePCR™ HotStar also contains the fluorescence dye, which is directly detected on BLooK LED Transilluminator (BK001) or UV epi-illuminator after the DNA electrophoresis. Ama*R* OnePCR™ HotStar mixture is supplied at the 2X concentration to allow 50% of the final reaction volume to be used for the addition of primer and template solutions. Reagents are provided with sufficient amplification reactions of 20 µl each.

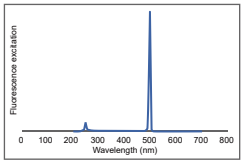


Fig. 1a Fluorescence excitation spectra of the fluorescence dye.

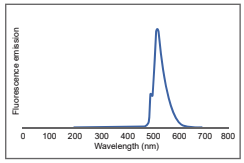


Fig. 1b Fluorescence emission spectra of the fluorescence dye.

## Features

- No post-staining processing of DNA required.
- No need to prepare PCR Reagents.
- Direct loading onto your agarose gel.
- Sensitivity – High degree of sensitivity as the ethidium bromide.
- Time efficiency – No destaining requirement.
- Compatibility –Use the UV light or blue light to detect the signal.
- Specificity – Reduce the non-specific amplification products, thus significantly improving the specificity of PCR reactions

## Application

- PCR Amplification

## Kit Content

| Catalog number | SM216-0250 | SM216-0005 |
|---|---|---|
| ama*R* OnePCR™ HotStar | 1.25 ml X 2 vials | 50 µl X 1 vial |

## Tracking Dye

- Amaranth

## Quality Control

The quality of the ama*R* OnePCR™ HotStar is tested on a lot-to-lot basis to ensure consistent product quality.

## Required Materials

- Electrophoresis equipments.
- DNA Markers (optional).
- BLooK LED Transilluminator or UV epi-illuminator

## Buffer Preparation

- TE buffer, pH8.0 (Selective): 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA

## Storage

Store at room temperature up to 1 month
Store at 4°C up to 6 months
Store at -20°C up to 1 year
Shipping temperature: 4°C

## ama*R* OnePCR™ HotStar Protocol

For each 20 µl reaction, assemble the following components in a 0.2 ml PCR tube on ice before the experiment:

| Component | Volume (µl) | Final Concentration |
|---|---|---|
| ama*R* OnePCR™ HotStar | 10 | 1X |
| Forward primer (5-10 µM ) | Variable | 0.1-0.2 µM |
| Reverse primer (5-10 µM) | Variable | 0.1-0.2 µM |
| DNA template | Variable | - |
| Add dd$H_2O$ to | 20 | |

2. Mix gently. If necessary, centrifuge briefly. Cap the tube and place it in the thermal cycler.
3. To process in the thermal cycler for 25-35 cycles as follows:

| Process | Temperature (°C) | Time | Cycles |
|---|---|---|---|
| Initial Denaturation | 94 | 2-5 minutes | 1 |
| Denaturation | 94 | 20-40 seconds | 25-35 |
| Annealing | the proper annealing temperature | 1 minute | |
| Extension | 72 | 2 minutes | |
| Final extension | 72 | 5 minutes | 1 |

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system.

4. After the PCR reaction, DNA electrophoresis will detect the PCR product.
5. Use the BLooK LED Transilluminator or UV epi-illuminator to photograph the gel.

Note: If the concentration of PCR amplification product is less than 4 pg, it may cause the migratory shift when performing the electrophoresis. To remedy this observation, we recommend to conduct the following Removal of fluorescence dye steps (please refer to the experimental procedures), or use the PCR Clean-Up & Gel Extraction Kit (catalog number: SN006-0100) to remove the fluorescence dye prior to post-staining with the Novel Green (catalog number: SL002-0500) or Novel Green plus (catalog number: SL003-0500) again for restoring the DNA molecular weight in the original position.

## Removal of fluorescence dye

1. Immerse the PCR product containing the fluorescence dye into the 100 mM NaCl and add 2.5 volumes of absolute or 95% ethanol.
2. Incubate on ice for 20 minutes.
3. Centrifuge the mixture at 4°C for at least 10 minutes.
4. Remove the suspension of ethanol and wash the pellet with 1ml of 70% ethanol.
5. Dry the residual ethanol and resuspend the double-stranded DNA in the TE buffer.

## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did PCR amplification with the kit.

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Refer to the table below to troubleshoot problems that you may encounter when you did PCR amplification with the kit.

| Problem | Cause | Solution |
|---|---|---|
| Low yield of PCR products | Incomplete concentration of start materials | Use the appropriate method for the DNA preparation based on the amount of the starting materials. |
| DNA degrade | DNA is not fresh | Avoid repeated freeze / thaw cycles of the sample. |
| | | Keep DNA preparations on ice or frozen in order to avoid the degradation. |
| | DNase contaminant | Use the fresh TAE or TBE electrophoresis buffer. |
| | | Maintain a sterile work environment to avoid contamination from DNase. |

## Related Ordering Information

| Cat. No. | Description | Size |
|---|---|---|
| SA001-0500 | AGAROSE Tablet, 0.5g | 100 Tablets |
| BK001 | BLooK LED Transilluminator | 1 Set |
| SD010-R600 | 1 Kb DNA Ladder RTU | 600 µl |
| SN005-0100 | Plasmid *mini*PREP Kit | 100 Reactions |
| SD110-0100 | OneMARK B | 600 µl |

## Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Ama*R* OnePCR™ HotStar is light sensitive and should be stored and protected from light.
- All products are for research use only.