ama*R* OnePCR™ HiFi

Cat. No.: SM215-0250 Size: 250 Reactions (2 X 1.25 ml)
Cat. No.: SM215-0010 Size: 10 Reactions (1X 100 µl)
Storage: Store at RT up to 1 months.
Store at 4°C up to 6 months.
Store at -20°C up to 1 year.
Note: ama*R* OnePCR™ HiFi is light sensitive and should be stored and protected from light.

Description

The ama*R* OnePCR™ HiFi is a ready-to-use PCR reaction mixture. Simply adding primers and template, the reagent will execute primer extensions and other molecular biology applications. The ama*R* OnePCR™ HiFi is a pre-mixed solution containing GDP-HiFi DNA polymerase, PCR buffer, dNTPs, gel loading dyes, enhancer, and fluorescence dye. It contains the fluorescence dye, which is directly detected on the blue-light transilluminator or UV epi-illuminator after the DNA electrophoresis. The GDP-HiFi DNA polymerase exhibits excellent processivity, elongation capability, and strong proof-reading activity. The elongation rate of this enzyme is approximately 2 times higher than that of *Taq* DNA polymerase. The elongation rate is 106-138 bases/s. and produces blunt end PCR products. The ama*R* OnePCR™ HiFi contents red tracking dyes, provide a safe, non-toxic and non-mutagenic alternative to ethidium bromide for instantaneous band visualization, that are environmentally friendly containing no hazardous chemicals. The tracking dyes that run at 10 bp on a 1% agarose gel. The ama*R* OnePCR™ HiFi mixture is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of the primer and template solutions. The reagents are provided with sufficient amplification reactions of 20 µl each.

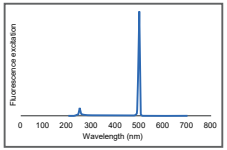

Fig. 1a. Fluorescence excitation spectra of the fluorescence dye

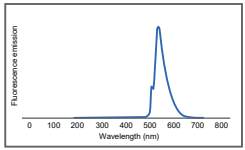

Fig. 1b Fluorescence emission spectra of the fluorescence dye

Features

- No post-staining procession.
- No need to prepare PCR reagents.
- Direct loading onto your agarose gel for analysis.
- Sensitivity- High degree of sensitivity as the ethium bromide.
- Time Efficiency- No destaining requirement.
- Compatibility - Use the Blue Light or UV to detect the signal.
- Effective for the amplification of GC-rich targets.
- Exhibits strong 3'→5' exonuclease activityTracking dyes

Tracking dye

- Amaranth

Protocol

Standard PCR with ama*R* OnePCR™ HiFi:

1. For each 20 µl reaction, assemble the following components in a 0.2 ml PCR tube on ice just prior to use:

Component	Volume (µl)	Final Concentration
ama*R* OnePCR HiFi™	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	4 pg~500 ng
Total	20	

2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in the thermal cycler.
3. Process in the thermal cycler for 25~35 cycles as follows:

Initial Denaturation	2~5 minutes at 94°C	
Denaturation	20~40 seconds at 94°C	30 cycles
Annealing	1 minute at the proper annealing temperature	
Extension	2 minutes at 72°C	
Final Extension	5 minutes at 72°C	

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

4. After the PCR reaction, DNA electrophoresis will detect the PCR product.
5. Use the UV or blue-light transilluminator or UV epi-illuminator to photograph the gel.

Note: When the DNA concentration 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 steps (please refer to the experimental procedures), or use the PCR Clean-Up & Gel Extraction Kit (see SN006-0100) to remove the flrorescence dye prior to post-staining with the Novel Green (SL002-0500) or Novel Green *plus* (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.

Related Ordering Information

Cat. No.	Description	Package
BK001	BLooK LED transilluminator	1 each
SL001-1000	Novel Juice	1 ml
SL003-0500	Novel Green *Plus*	500 µl
SM213-0250	ama*R* OnePCR™	250 Reactions
SM216-0250	ama*R* OnePCR™ HotStar	250 Reactions
SD101-0100	OneMARK 100	600 µl
SD110-0100	OneMARK B	600 µl

Caution:

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. All products are for research use only.