Absolute Master Mix 2

Cat No: AM-M-002-1250
Size: 1.25ml
Stored at 25°C up to one week, at 4°C up to six months and at -20°C up to one year.
The product is light sensitive therefore protect from light

MOLEQULE-ON®

Description

Absolute Master Mix 2 is a ready-to-use PCR reaction mixture. It only requires addition of primers, DNA template and water to carry out polymerase chain reaction. Absolute Master Mix 2 contains *Taq* DNA polymerase, PCR Buffer, dNTPs, loading dyes enhancer and fluorescence dye. Absolute Master Mix 2 is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer and template solutions. Components of Absolute Master Mix 2 are tested for the absence of DNase, RNase and exonuclease activities. This ready mix contains the fluorescence dye is directly detected on LED transilluminator or UV illuminator after the DNA electrophoresis.

Features

- No post-staining processing of DNA required.
- Direct loading onto agarose gel for electrophoresis.
- High degree of sensitivity equivalent to ethidium bromide.
- Benefit for quick set up of PCR reaction such as colony PCR when screening for positive clones.
- Compatible with both blue light or UV to detect the signal.

Protocol

Standard PCR with Absolute Master Mix 2:

1. For each 50 µl reaction, add the following materials in a 0.2 ml PCR tube on ice just prior to use:

Absolute Master Mix 2	25µl
Forward primer, 5-10 µM	1µl
Reverse primer, 5-10 µM	1µl
DNA template	1µl
Add ddH_2O to make volume up to	50µl

2. Mix gently and briefly centrifuge.
3. Cap the tubes tightly to avoid expulsion and evaporation.
4. Place the tubes in thermal cycler and process according to recommended thermal cycler conditions; initial denaturation at 94°C for 3-5 minutes, 30-40 cycles of denaturation at 94°C for 30 seconds, annealing (Tm of primer – 5) for 1 minute, extension at 72°C for 2 minutes and 1 cycle of final extension at 72°C for 7-15 minutes.

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.

5. After the PCR reaction, run on agarose electrophoresis to detect PCR product. No additional dye is required for the PCR samples.
6. Use the UV or blue-light to visualize the gel.

Removal of fluorescence dye

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

Caution

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. Not intended for any animal or human therapeutic or diagnostic uses.