ABamp basic Kit for DNA

Cat. No. : RK20738

Product components

Components	Component number	Size-1 16 RXN
2×Reaction Buffer	RM20825	600 μL
MgOAC	RM20826	60 μL
RPA PCR reagent	RM20827	16 RXN

Product Description

Recombinase polymerase amplification (RPA) is a novel nucleic acid amplification technique (NAAT) that enables rapid amplification of targets at a constant low temperature (37~42℃); The target nucleic acid molecules can be amplified to detectable levels within 10-30 minutes. This kit helps user acheive high detection sensitivity, specificity, and short reaction time (only 15-25 minutes). The reaction components are in the form of lyophilized beads, which are easy to operate and store. This reagent can be used for reaction in metal baths, water baths, etc., and user doesn't need to purchase other expensive devices.

Storage Conditions

Transportation conditions: Ice pack transportation;

Storage conditions: Store at a temperature of -15 to -25 ℃ in dark and dry place, and avoid repeated freeze-thaw cycles;

Limit of detection

The minimum detection limit of this kit is 10-100 copies/t ,which depend on primer selection optimization and detection method.

Primer design

It is recommended to use primers with a length of 30-35 bp, as too short primers will affect the amplification speed and detection sensitivity; Primer design should avoid causing formation of secondary structures; The amplicon length is recommended to be between 100-1000 bp and usually not more than 1000 bp.

Protocol

1. Take out the required components for 20 minutes in advance, thaw at room temperature, shake and mix well.
2. Add 25 μL 2×Reaction Buffer to each bead reaction tube.
3. Add 2 μL upstream primers and 2 μL downstream primer.
4. Add 18.5-x μL ddH2O and x μL ($2.5 \leq x \leq 10$) nucleic acid template.
5. Finally, add 2.5 μL MgOAC to the reaction tube and mix thoroughly.

Note: Please make sure to invert the reaction tube for 5 seconds to mix well; for multiple reactions, it is recommended to add MgOAC to the inside of the reaction tube cover, centrifuge together, and shake well.

5. Mix well, rapidly centrifuge, and then immediately place the reaction tube in a constant temperature equipment and incubate at 37-39 ℃ for 30 minutes.
6. After the reaction is complete, add 50 μL phenol/chloroform to extraction. 12000 rpm, 5 minutes, then take 5 μL supernatant to detect through agarose gel electrophoresis.

Optional: Use magnetic beads for purification, and then take 5 μL was detected by agarose gel electrophoresis.

Reagent	volume（μL）
2×Reaction Buffer	25
upstream primer（10μM）	2
downstream primer（10μM）	2
ddH2O	18.5-x
template	2.5＜x＜10
MgOAC	2.5
Total Volume	50

Precautions

1. In order to avoid cross-contamination, the reagent preparation area and the PCR amplification analysis area should be separated.

2. A blank control without template should be set up during the experiment to confirm whether there is contamination of nucleic acids to be amplified.

3. Under different nucleic acid extraction methods, the DNA content and purity of the extracted samples will be different, which may lead to different amplification efficiency (such as PCR inhibitors: ethanol, phenol, heme, etc.).

4. It is recommended to add 2.5μL of positive standard, and sample amount varies in a ranges of 2.5μL~10μL. If the sample concentration is high, 2.5 μL is enough, otherwise, the sample amount is increased to a maximum volume of 10 μL.