Multienzyme Isothermal Rapid Amplification

RT- Basic kit (RNA)-II

Guide Manual

（Part number：WLRB8207KIT）

Overview of the MIRA technology

Multienzyme isothermal rapid amplification(MIRA) is based on a combination of polymerases and DNA recombination/repair proteins: Reverse transcriptase generate DNA from RNA template. Recombinases bind to single-stranded nucleic acid and stimulate the resulting protein-DNA complex to search for homologous sequences in duplex DNA with Single Strand Binding protein. Once homology is located, a strand-switching reaction is performed and the oligonucleotide is paired to its complement permitting a polymerase to begin synthesis from the 3’ end. The amplification process is very rapid when optimised and can reach detectable levels of product in a few minutes.

Primer design considerations

The recommended primer length is 30 to 35 nucleotides . It is best to avoid unusual sequence elements within the primer, such as long tracks of one particular nucleotide or a large number of small repeats. oligonucleotides that contain sequence elements that promote secondary structures and primer-primer interactions or hairpins should be discarded.The recommended amplicon length is not exceed 500 bp, and ideally is between 150 -300 bp .

Materials provided

Materials provided	Content
A buffer	1 tube × 1.6 mL
B buffer	1 tube × 150 μL
Reaction unit	48
Guide manual	1

Storage conditions

- Transport temperature: ≤ 20 ºC constant temperature environment.
- Store at -20 ºC without light， no repeated freezing and thawing.
- Full activity is guaranteed for 14 months.

Set up

1. Add 29.4 μL A buffer (supplied) to a MIRA basic reaction.
2. Add 2 μL forward primer and 2 μL reverse primer (Primer concentration: 10 μM).
3. Add template and water (template and water total volume is 14.1 μL).
4. Add 2.5 μl B buffer (supplied) and mix well, then spin briefly to start reaction.

5. Incubate at 42˚C for 30 minutes.
6. Add Tris saturated phenol/chloroform/isoamyl alcohol (25:24:1), and mix 1:1 to reaction solution, centrifuge at 12000 rpm for 5 min, take 5 μL of supernatant for1.5% -2% agarose gels agarose.

Reaction mix

A buffer	29.4 μL
Forward primer (10 μM)	2 μL
Reverse primer (10 μM)	2 μL
RNA template and water	14.1 μL
B buffer	2.5 μL
Total volume	50 μL

Notes

1. Reactions start as soon as B buffer is added.
2. Set up a negative control for each experiment.
3. For research and development use only.

Ordering information and technical support

Amp-Future (Changzhou) Biotech Co., Ltd.
4th Floor, No.9 Building, China Israel Changzhou Innovation Park (CICP),
Changzhou City
Jiangsu Province
China