Multienzyme Isothermal Rapid Amplification

NFO-Kit (DNA)

Guide Manual

（Part number：WLN8203KIT）

Overview of the MIRA technology

Multienzyme isothermal rapid amplification(MIRA) is based on a combination of polymerases and DNA recombination/repair proteins: 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, 2 μL reverse primer and 0.6 μL probe(primer/probe concentration: 10 μM).
3. Add template and water (template and water total volume is 13.5 μL).
4. Add 2.5 μl B buffer (supplied) and mix well （Turn the tube upside down for 8-10 times to mix well）and spin briefly to start reaction.

5. After blending to force (or use centrifuge) the mixture to the bottom of a test tube. Then incubate at 39˚C for 8-12 minutes by water bath or dry bath.
6. Add 10 μl reaction solution into the centrifuge tube which containing 190 μl ddH_2O. After mixing, insert the sample end of colloidal gold test strip into the centrifuge tube，Read the results of control line and test line in 5 mins.

Reaction mix

A buffer	29.4 μL
Forward primer (10 μM)	2 μL
Reverse primer (10 μM)	2 μL
DNA template and water	13.5 μL
Probe (10 μM)	0.6 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

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