

Multienzyme Isothermal Rapid Amplification

RT-nfo-Kit (RNA)-II

Guide Manual

(Part number: WLRN8209KIT)

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 primers length are 30-35 bp to avoid the speed and detection sensitivity. The 5' end of the downstream primer is labeled with a modification group (usually biotin), and mind secondary structures when designing the primer, recommended primer length is 150-500bp.

Materials provided

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

- Transport temperature: ≤ 20 °C constant temperature environment.
- Store at -20 °C 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 42°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 ddH₂O. 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
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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