Multienzyme Isothermal Rapid Amplification

RT-exo Kit (RNA)-II

Guide Manual

（Part number：WLRE8208KIT）

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. The kit relies on the role of exonuclease and adds the designed specific molecular probes according to the template at 42℃， achieves the real-time monitoring of amplification process of the target fragment. And it is suitable for laboratory RNA amplification and RNA amplification for other detection purposes.

Primer design considerations

The recomended 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.

Probe design considerations

The probe sequence does not overlap with the primers recognition site and should be 46-52 nucleotides long. The probe of an oligonucleotide backbone that contains: A dSpacer (tetrahydrofuran, THF) is as a recognition site for the exonuclease , which at least 30 bases are placed 5’ to it, and at least a further 15 bases are located 3’; a flanking dT-fluorophore (typically fluorescein, but any fluorophore available as dT-coupled reagents for oligonucleotide synthesis can be used). The distance between fluorescence group and quenching group is 3-5 bases, located on both sides of THF group.

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 ºC.

- 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, then spin briefly to start reaction.
5. Place reactions in a fluorometer and start run: 39°C, 20 minutes, detect fluorescent signal every 30s.

Reaction mix

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