RPApex Basic: Recombinase Aided Amplification Kit

Cat. No. JY0800

Principle

RPApex Basic uses enhanced Recombinase Aided Amplification (RAA) technology for isothermal amplification at 39–42℃.

- Recombinase-primer complex invades target DNA template
- Single-strand binding proteins maintain open-chain state
- DNA polymerase initiates strand synthesis at 3′ end

- **Exponential amplification achieved in 20–40 minutes**
  - Ready-to-use lyophilized pellets for easy transport and storage

Primer Design Guidelines

- Length: 28–35 nt (longer than PCR primers)
- Too short (<28 nt): reduced recombinase binding efficiency
- Too long (>35 nt): secondary structures (hairpins, dimers) interfere with specificity

- **Concentration: optimize via gradient experiments (0.2–0.6 μM)**
  - Amplicon length: 80–500 bp recommended
  - GC content: 40–60% preferred
  - Verify specificity via BLAST to avoid cross-reactivity

Intended Use

1. **For research use only (RUO)**
   1) **Amplification of DNA and RNA templates**
   - Applications: CRISPR detection, agarose gel electrophoresis analysis

Materials Supplied

- 48 reactions per kit
- Lyophilized pellets containing: recombinase, DNA polymerase, SSB, ATP, dNTPs, buffer components

2) **Note: Mg^{2+} not included by default. Recommended final concentration: 14 mM/Test. Specify when ordering if required.**

Storage and Stability

2. **Unopened: Store at 25 – 30℃, dry and light-protected. Shelf life: 12 months (≥95% activity retention)**

- After opening: Tighten cap, continue storage at 25–30℃, dry and light-protected. Shelf life: 2 months

Component	Volume
Primer F (10 μM)	1 μL
Primer R (10 μM)	1 μL
Enzyme Lyophilized Pellet	1 pellet
Mg^{2+}	1 Mg^{2+} pellet OR 1.25 μL of 280 mM Mg^{2+}
Nucleic Acid Solution or Crude Lysate	2–4 μL
Nuclease-free Water	Bring to 25 μL total volume

3. **Sample Preparation**

Animal Samples (muscle or organ: spleen, tonsils, lymph nodes, kidneys, bone marrow):

- Cut 0.05 g or 3 mm^3 tissue pieces into centrifuge tube
- Option 1: Add 200 μL of 5× Lightning Reagent (BT0066), heat at 95℃ for 5 min, use supernatant
- Option 2: Add 50–200 μL of CoolFlash (BT0068) or StarFlash (BT0069) Reagent

4. **Plant Samples (leaf, root, stem, or bud):**
   - Cut 2–3 mm^2 pieces into centrifuge tube

5. **• Add 50–200 μL of CoolFlash (BT0068) or StarFlash (BT0069) Reagent**
   - Grind briefly, vortex to mix, use supernatant

6. Reaction Setup

Pipetting order: Negative control → Test samples → Positive control

7. **Cap immediately after each addition to avoid aerosol contamination.**

Mixing

8. **• Vortex at low speed or pulse mode for 3 – 5 seconds, OR**
   - Cap tightly and invert 3–5 times

• Centrifuge briefly and proceed to reaction

Incubation

• Place tubes at 39–42℃ for 20–40 minutes

1. Post-Reaction Processing
2. 1. Remove tubes from incubator immediately after reaction.
3. 2. Add 50 μL phenol/chloroform (1:1 v/v) to each tube.
4. 3. Vortex vigorously for 10–15 seconds.
5. 4. Centrifuge at 12,000 rpm for 1 minute to separate phases.
6. 5. Pipette 10 μL of supernatant (aqueous phase) for agarose gel electrophoresis.

6. Recommended gel concentration: 1.5%–2.0% (adjust based on amplicon size).

7. Visualize using appropriate staining (e.g., EB or GelRed).

Detection Sensitivity