

**Principle**

RPApex Fluorescence/LFS uses enhanced RAA technology for isothermal amplification at 39 – 42°C.

- Recombinase-primer complex invades target DNA
- SSB proteins maintain open-chain state
- DNA polymerase initiates strand synthesis
- Exponential amplification in 10 – 20 minutes
- Contains exonuclease/Nfo nuclease for fluorescence or LFS detection

• **Ready-to-use lyophilized pellets**

**Primer Design Guidelines**

- Length: 28 – 35 nt (longer than PCR primers)

• **Too short (<28 nt): reduced efficiency**

- Too long (>35 nt): secondary structures interfere
- Optimize concentration via gradient (0.2 – 0.6 μM)

**Probe Design**

- Length: 28 – 35 nt, complementary to target

• **Introduce THF (dSpacer) for enhanced cleavage**

1. • **Fluorescence: 5' fluorophore (FAM), 3' quencher (BHQ1)**

1) • **LFS: 5' antigen tag (FAM), 3' blocking group (C3-spacer)**

- Avoid secondary structures; verify with Primer Premier or OligoAnalyzer

2) **Amplicon Length**

- Recommended: 80 – 300 bp

2. • **Too long (>300 bp): reduced efficiency**

- GC content: 40 – 60% preferred

Component	Volume
Primer F (10 μM)	1 μL
Primer R (10 μM)	1 μL
Probe (10 μM)	0.25 μL
Enzyme Lyophilized Pellet	1pellet
Mg <sup>2+</sup>	1 magnesium pellet or 280 mM Mg <sup>2+</sup> 1.25 μL
Nucleic Acid Solution or Crude Lysate	2 ~ 4 μL
Add water to make up volume to	25 μL

3. • **Verify specificity via BLAST**

4. **Intended Use**

5. • **For research use only**

6. • DNA and RNA template amplification

• Detection: fluorescence reader or lateral flow strips (LFS)

Note: Products NOT suitable for agarose gel electrophoresis or CRISPR/Cas analysis.

### Materials Supplied

1. • 48 tests per kit
2. • Lyophilized pellets with recombinase, polymerase, exonuclease/Nfo, dNTPs, buffer
3. Note: Mg<sup>2+</sup> not included. Recommended: 14 mM/Test. Specify when ordering.
4. Storage and Stability
5. • Unopened: 25 - 30°C, dry and light-protected. Shelf life: 12 months (≥95% activity)
6. • After opening: Tighten cap, 25 - 30°C, dry and light-protected. Shelf life: 2 months

### Sample Preparation

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