

## Nucleic Acid Extraction-Free Kit

Cat. No.: N1508469 | Pack size: 50 tests | Storage: Room temperature

### Overview

This kit uses a patented buffer system containing Lysis Buffer B1 and Lysis Buffer B2 for direct lysis of DNA or RNA viruses. It is suitable for direct molecular detection of DNA or RNA viruses from blood and tissue sample materials.

The extraction-free workflow does not require protease digestion, high-temperature incubation, deproteinization, organic solvent extraction, or ethanol precipitation. The process is simple, rapid, stable, and suitable for direct PCR/qPCR or RT-qPCR template preparation.

### Key Features

- Extraction-free workflow with viral DNA or RNA release completed in approximately 5 min
- No liquid nitrogen, organic extraction, or ethanol precipitation required
- Suitable for tissue materials, blood, plasma, and tissue homogenates
- Compatible with downstream qPCR and RT-qPCR amplification systems

### Contents & Storage

| Cat. No.  | Component       | Size   | Storage |
|-----------|-----------------|--------|---------|
| N1508469A | Lysis Buffer B1 | 5.5 mL | RT      |
| N1508469B | Lysis Buffer B2 | 5.5 mL | RT      |

### Materials Required But Not Supplied

| Item                        | Recommended Specification                                                      | Purpose                   |
|-----------------------------|--------------------------------------------------------------------------------|---------------------------|
| Sample material             | Tissue sample 5-10 mg, plasma, homogenate, or fresh anticoagulated whole blood | Nucleic acid release      |
| 1.5 mL centrifuge tubes     | Clean, nuclease-free                                                           | Sample processing         |
| Tissue grinder              | For tissue samples                                                             | Mechanical homogenization |
| Vortex mixer and centrifuge | 10,000 rpm capability                                                          | Mixing and clarification  |

| Item                                      | Recommended Specification           | Purpose          |
|-------------------------------------------|-------------------------------------|------------------|
| Direct amplification PCR/RT-qPCR reagents | User-validated amplification system | Result detection |

## Preparation Before Use

1. Bring reagents to room temperature and mix gently. Use clean nuclease-free consumables. Prepare lysates immediately before amplification.

## Protocol

### Sample Amount Guidance

The recommended tissue input is 5-10 mg, approximately the size of one to half a grain of rice. Excessive sample input may cause false-negative results.



Reference image for tissue sampling amount: 100 mg, 50 mg, and 10 mg examples.

### Tissue Samples

Place 5-10 mg tissue sample into a 1.5 mL centrifuge tube. Add 100  $\mu$ L Lysis Buffer B1, ensure the buffer covers the sample, and grind thoroughly. Leave at room temperature for 5 min. Add 100  $\mu$ L Lysis Buffer B2, vortex to mix, and centrifuge at 10,000 rpm for 2 min. Carefully transfer 100  $\mu$ L supernatant to a clean tube as template. Use approximately 1/10 of the PCR reaction volume as template (e.g., 5  $\mu$ L template in a 50  $\mu$ L PCR reaction).

**Note:** The template must be prepared and used immediately.

### Blood Samples

Add 20  $\mu$ L (or 10  $\mu$ L) tissue homogenate, plasma, or fresh anticoagulated whole blood to 100  $\mu$ L Lysis Buffer B1. Vortex and leave at room temperature for 5 min. Add 100  $\mu$ L Lysis Buffer B2, vortex to mix, and centrifuge at 10,000 rpm for 2 min. Transfer 100  $\mu$ L supernatant to a clean tube as template. Use approximately 1/10 of the PCR reaction volume as template.

**Note:** The template must be prepared and used immediately.

### Recommended qPCR Amplification System

| Component                                    | Volume      |
|----------------------------------------------|-------------|
| ddH <sub>2</sub> O                           | Up to 50 µL |
| Direct Amplification qPCR Reaction Mix       | 38 µL       |
| Direct Amplification HotStart Taq Enzyme     | 2 µL        |
| Direct Amplification qPCR Primers and Probes | 5 µL        |
| Template                                     | 5 µL        |

### Primer-Probe Mix for qPCR (Recommendation)

| Primer-Probe | Stock Solution | Final Concentration in Mix | Single-Serve Volume |
|--------------|----------------|----------------------------|---------------------|
| Primer F     | 40 pmol/µL     | 4 pmol/µL                  | 5 µL                |
| Primer R     | 40 pmol/µL     | 4 pmol/µL                  | 5 µL                |
| TaqMan Probe | 20 pmol/µL     | 2 pmol/µL                  | 5 µL                |

### qPCR Reaction Program

| Step | Temperature                             | Time        | Number of Cycles |
|------|-----------------------------------------|-------------|------------------|
| 1    | 95 °C                                   | 3 min       | 1 cycle          |
| 2    | 95 °C / 60 °C (fluorescence collection) | 15 s / 30 s | 40 cycles        |

Annealing temperature should be determined according to primer and probe design.

### Recommended RT-qPCR Amplification System

| Component          | Volume      |
|--------------------|-------------|
| ddH <sub>2</sub> O | Up to 50 µL |

| Component                                       | Volume     |
|-------------------------------------------------|------------|
| Direct Amplification RT-qPCR Reaction Mix       | 38 $\mu$ L |
| Direct Amplification RT-qPCR Enzyme Mix         | 2 $\mu$ L  |
| Direct Amplification RT-qPCR Primers and Probes | 5 $\mu$ L  |
| Template                                        | 5 $\mu$ L  |

### RT-qPCR Reaction Program

| Step | Temperature                             | Time        | Number of Cycles |
|------|-----------------------------------------|-------------|------------------|
| 1    | 50 °C                                   | 10 min      | 1 cycle          |
| 2    | 95 °C                                   | 3 min       | 1 cycle          |
| 3    | 95 °C / 60 °C (fluorescence collection) | 10 s / 30 s | 40 cycles        |

### Storage & Handling

Store at room temperature for up to 24 months. Keep containers tightly closed and avoid contamination.

### Safety & Precautions

1. This kit does not have independent in vitro diagnostic function and must not be used as a diagnostic reagent.
2. Strictly control sample input. Excessive tissue input may inhibit amplification or cause false-negative results.
3. Use prepared template immediately and avoid repeated freeze/thaw or prolonged storage of lysates.
4. Wear lab coat, gloves, and eye protection. Read the current SDS before use.

## Quality Control

| QC Item                | Method                                       | Acceptable Range                                                                   |
|------------------------|----------------------------------------------|------------------------------------------------------------------------------------|
| Component completeness | Visual inspection on receipt                 | All listed components present; no leakage or visible damage                        |
| Enzyme performance     | Functional amplification or end-repair assay | Meets product specification when used under recommended conditions                 |
| Storage integrity      | Cold-chain and label check                   | Stored at the specified temperature and protected from repeated freeze/thaw cycles |

## Troubleshooting

| Issue                         | Possible Causes                                        | Corrective Action                                                                 |
|-------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------|
| False-negative or weak signal | Excessive tissue input or inhibitors in lysate         | Use 5-10 mg tissue, clarify lysate, and limit template to 1/10 of reaction volume |
| Poor reproducibility          | Incomplete homogenization or inconsistent sample input | Standardize sample amount and grinding process                                    |
| Amplification inhibition      | Too much lysate in PCR/qPCR                            | Reduce lysate input or perform dilution optimization                              |

## Recommended Applications

Extraction-free nucleic acid release · direct qPCR · direct RT-qPCR · viral DNA/RNA detection research

## Contact & Global Offices

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## Limitations & Disclaimer

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