Lumiprobe
Life science solutions

LumiPure® UNI, Precipitation-Based Nucleic Acid Isolation Kit for Any Sample manual

Contents

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LumiPure® UNI, Precipitation-Based Nucleic Acid Isolation Kit for Any Sample manual

The kit is designed for total nucleic acid extraction from a variety of samples including plant and animal tissues, organs, blood plasma, myeloblasts, mammalian cell cultures, Gram-negative bacterial cultures, epithelial cell swabs, smears, washing fluids, and other liquid biological samples. The total nucleic acid isolated with the kit is compatible with the downstream PCR or RT-PCR.

Kit components

Kit component	Count 34663 100 assays
P7450, Lysis Solution NA, 30 mL	1
R7050, Precipitation Solution, 40 mL	1
S8150, Wash Solution 1, 50 mL	1
S6050, Wash Solution 2, 50 mL	1
G5850, Dissolving buffer NA, 5 mL	1

Store at temperature between +2 °C and +8 °C. Transportation: for up to 3 weeks at 30 °C or at temperatures up to+2 °C and +8 °C within the entire shelf life.

Shelf life 12 months.

Hardware and Consumables Required but not Supplied:

- Dry Block Heater (or water bath);
- Centrifuge that accommodates 1.5 mL tubes, and is capable of generating at least 13,000 rpm (11,000 × *g*);
- 1.5 mL microcentrifuge tubes (1-2 tubes for extraction from 1 sample);
- Additional materials (depending on sample type):
    - *tissue samples, plant or animal:* liquid nitrogen, mortar and pestle set
    - *cell cultures, plant or animal:* Phosphate-Buffered Saline (PBS)
    - *liquid biosamples, swabs, washing fluids, feces:* sterile saline
    - *sputum:* mucolysin

Before You Begin

If the *Lysis Solution NA* contains a precipitate, heat the buffer to 50 °C in the heater and wait for the precipitate to dissolve completely.

Sample Preparation

Plant and animal tissues

20-30 mg of sample weight is recommended. Both fresh and frozen samples can be used.

! Samples can be stored at -70 °C for several months.

1. Place a fresh or a frozen (-70 °C) tissue sample into liquid nitrogen.
2. Transfer the frozen sample to a mortar and thoroughly homogenize it to powder.
3. Transfer the powder into a separate 1.5 mL tube. Wait for liquid nitrogen to evaporate but do not allow the powder to thaw.
4. Add the following reagents to the homogenate in the following order: 300 µL of *Lysis Solution NA*, 100 µL of water. Mix the contents.
5. Proceed to step 2 of **'Nucleic Acid Extraction'**.

Animal or bacterial cell cultures

Samples of no more than 1-2×10^6 cell counts for animal cell cultures or 10^9 cell counts for Gram-negative bacterial cell cultures are recommended.

Adherent cell culture: remove media, harvest cells with trypsin (or with other methods recommended for the cell culture used). Centrifuge the sample at 300 × *g* for 5 min. Discard the supernatant. Resuspend the cell pellet in 100 µL of PBS. Transfer the suspension into a new 1.5 mL tube.

Animal cells in suspension culture: collect the suspension culture volume required to obtain the desired cell number. Centrifuge cells at 300 × *g* for 5 min. Discard the supernatant. Resuspend the cell pellet in 100 µL of PBS. Transfer the suspension into a new 1.5 mL tube.

Bacterial cell culture: collect the bacteria grown with liquid or solid media with

centrifugation at 3,000-5,000 × *g* for 5-10 min. Discard the supernatant. Resuspend the cell pellet in 100 µL of PBS. Transfer the suspension into a new 1.5 mL tube.

Proceed to **'Nucleic Acid Extraction'**.

Blood Plasma

This kit is suitable for nucleic acid extraction from blood plasma. Plasma must be collected from peripheral whole blood samples containing EDTA (2.0 mg/mL) or citrate as an anticoagulant.

! Heparin must not be used as an anticoagulant.

! Plasma must be obtained within 6 hours from peripheral blood sample collection.

1. Mix the blood sample by the vial inversion to ensure adequate homogenisation.
2. Centrifuge the vial with the blood sample at 900 × *g* for 20 min at room temperature (18-25 °C).
3. Aspirate 100 µL of the supernatant (plasma) and transfer to a separate 1.5 mL tube.
4. Proceed to **'Nucleic Acid Extraction'**.

! Store the plasma sample at -20 °C for no more than 3 months.

Epithelial cells in swab samples

This kit is suitable for total nucleic acid extraction from swab samples of epithelial cells collected with a single-use sterile swab (buccal, posterior pharynx, nasopharyngeal, urethral, cervical, vaginal swabs, etc.).

1. Place 500 µL of sterile saline into a 1.5 mL tube.
2. Vigorously swirl the swab to resuspend the sample material in saline. Press the swab against the wall of the vial and squeeze out the residual saline with a circular motion.

3. Centrifuge the solution at 11,000 × *g* for 10 min. Thoroughly remove the supernatant.
4. Resuspend the pellet in 100 µL of sterile saline.
5. Proceed to **'Nucleic Acid Extraction'**.

Urine

1. Transfer 1 mL of a urine sample into a clean 1.5 mL tube.
2. Centrifuge at 11,000 × *g* for 10 min. Thoroughly remove the supernatant.
3. Resuspend the pellet in 1 mL of sterile saline.
4. Centrifuge at 11,000 × *g* for 10 min. Discard the supernatant.
5. Resuspend the pellet in 100 µL of sterile saline.
6. Proceed to **'Nucleic Acid Extraction'**.

Saliva, liquor, synovial fluid

1. Add 500 µL of the sample into a separate 1.5 mL tube.
2. Centrifuge at 11,000 × *g* for 10 min. Thoroughly remove the supernatant leaving ~50 µL of the solution above the pellet.
3. Resuspend the pellet in 500 µL of sterile saline.
4. Centrifuge at 11,000 × *g* for 10 min. Discard the supernatant.
5. Resuspend the pellet in 100 µL of sterile saline.
6. Proceed to **'Nucleic Acid Extraction'**.

Semen, prostate secretes

1. Add 100 µL of the sample into a separate 1.5 mL tube.
2. Add 500 µL of sterile saline into the tube, vortex for 5-10 sec.
3. Centrifuge at 11,000 × *g* 10 min. Discard the supernatant.
4. Resuspend the pellet in 100 µL of sterile saline.
5. Proceed to **'Nucleic Acid Extraction'**.

Smears and washing fluids

1. Place the specimen to the centrifugation vial.
2. Centrifuge at 11,000 × *g* 10 min. Discard the supernatant.
3. Resuspend the pellet in 100 µL of sterile saline.
4. Proceed to **'Nucleic Acid Extraction'**.

Feces

1. Transfer 1 mL of sterile saline into a clean 1.5 mL tube.
2. Add ~250 mg (µL) of feces into the tube.
3. Vortex the content for 5-10 sec.
4. Centrifuge at 100 × *g* for 3 min.
5. Transfer 800-1,000 µL of supernatant into a separate 1.5 mL tube.
6. Centrifuge for at 11,000 × *g* 10 min. Discard the supernatant.
7. Resuspend the pellet in 100 µL of sterile saline.
8. Proceed to **'Nucleic Acid Extraction'**.

Sputum specimen

1. Add mucolysin to a vial containing the sample in the ratio of 5:1 (5 parts mucolysin and 1 part of sputum) using graduated marks on the vial.
2. Close the vial lid, shake the content. Incubate for 20-30 min at room temperature, shake the vial every 2-3 min.

   ! The processed sputum sample can be stored at 4 °C for 24 hours or at -20 °C for long-term.
3. Transfer 500 µL of the mucolysin-treated sputum sample into a separate 1.5 mL tube.
4. Centrifuge for at 11,000 × *g* 10 min. Discard the supernatant.
5. Resuspend the pellet in 100 µL of sterile saline.
6. Proceed to **'Nucleic Acid Extraction'**.

Nucleic Acid Extraction

All the procedures should be performed at room temperature; >13,000 rpm (>11,000 × *g*) centrifugation should be used unless directed otherwise.

Before you begin, set the dry block heater to 65 °C and preheat the vial with *Resuspension Buffer NA*.

Prepare the specimen in 100 µL volume in a 1.5 mL tube as directed in **'Sample Preparation'**.

1. Add 300 µL of *Lysis Solution NA* to the vial containing the sample (100 µL), and mix thoroughly by vortexing.
2. Incubate the resulting solution at 65 °C for 15 min.
3. *(Optional)* If the sample is not fully dissolved after lysis, centrifuge the vial for 10 min. Transfer the supernatant into a new 1.5 mL tube.
4. Add 400 µL of *Precipitation Solution* to the vial containing the sample and vortex. Centrifuge for 15 min.
5. Carefully remove the supernatant, take care to avoid disturbing the pellet. Add 500 µL of *Washing Solution 1* to the pellet and mix by vortexing. Centrifuge for 5 min.
6. Carefully remove the supernatant, take care to avoid disturbing the pellet. Add 500 µL of *Washing Solution 2* to the pellet and mix by vortexing. Centrifuge for 5 min.
7. Thoroughly remove the supernatant, avoid disturbing the pellet. Open the vial and dry the pellet at 65 °C for 5 min.
8. Add 50 µL of preheated *Resuspension Buffer NA*.
9. Incubate the vial containing the specimen at 65 °C for 5-10 min. Mix the content by vortexing and centrifuge to collect drops. The resulting product of total nucleic acid is suitable for downstream PCR or RT-PCR without additional processing.

Extracted RNA Storage: *We do not recommend storing extracted RNA due to its instability.* The extracted RNA product must be used in the downstream reverse transcription-polymerase chain reaction immediately.

Extracted DNA Storage: Store at 4 °C for short-term; store at -20 °C for no more than 1 month or at -70 °C for no longer than 1 year.

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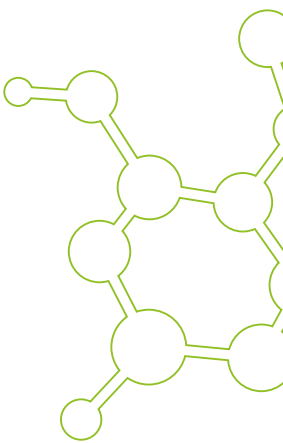

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