Nucleic Acid Isolation Kit

Cat. No.:	YNR100	YNR500
Product Name:	Nucleic Acid Isolation Kit	
Reactions:	100	500
Yield:	Up to 30ug for 3 ml of cultured bacterial cells, 30ug for 5 x 10^6 cultured animal cells, 80ug for 50mg of tissue.	
Format:	Reagent	
Operation:	Centrifuge	
Operation Time:	Within 90 Minutes	

Description

Nucleic Acid Isolation Kit provides a fast and simple method to isolate total DNA and RNA from fresh blood, serum, cultured animal cells, cultured bacteria cultures and tissue. The scalable purification procedure gently removes contaminants and inhibitors and allows large-volume samples to be purified for use as long-term references. The unique NR Buffer system ensures total Nucleic Acid with high yield and good quality from samples of unlimited size. If a larger sample is required, the buffer volume can be scaled proportionately.

When extracting Nucleic Acid from fresh blood samples, RBC Lysis buffer is required. Even though this kit is designed specifically for total Nucleic Acid isolation, RNase A or DNase I can be applied accordingly while isolating only total DNA or total RNA is required. The entire procedure can be completed in 1 hour. DNA phenol extraction is not required. The total DNA/RNA is ready for use in PCR, RT-PCR, Southern Blotting, Northern Blotting, Mapping and RFLP.

Features

1. Convenient, scalable purification procedure.
2. Reproducible recoveries for constant results.
3. Ready-to use total DNA/RNA within 1 hour.

Contents

ITEM	YNR100	YNR500
NR Buffer	100ml	500ml

*If NR Buffer contains sediment, incubate at 65ºC for 10 minutes to dissolve.

Applications

Purified total DNA/RNA is ready for direct use in PCR, RT-PCR, Southern Blotting, Northern Blotting, Mapping and RFLP.

Quality Control

The quality of the Nucleic Acid Isolation Kit is tested on a lot-to-lot basis by isolation of total Nucleic Acid from 50 mg of tissue samples. A minimum of 20 µg of total Nucleic Acid can be quantified with a spectrophotometer and checked by electrophoresis.

Caution

NR Buffer contains irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Protocol

Additional Requirements:

Microcentrifuge tubes, Absolute Ethanol for preparing 70% Ethanol in water, Chloroform, Isopropanol, Mortar and Pestle.

Optional requirements:

If a larger sample volume is required, scale the NR Buffer proportionately. For RNA degradation, add RNase A (10 mg/ml) according to the protocol in the Step 1 Lysis. For DNA degradation, add 2 µl of DNase I (2 KU/ml), mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the final sample in the Nucleic Acid Precipitation Step. Let stand for 10 minutes at room temperature.

Tissue Dissociation or Cultured Cell Harvesting or RBC Lysis	**Tissue** ★Cut off 50 mg of fresh tissue. ★Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.
	Cultured Animal/Bacterial Cells ★Transfer cultured animal cells (up to 5 x 10^6) or 1.5 ml of bacterial culture to a microcentrifuge tube. ★Microcentrifuge for 1 minute and pour out the majority of the supernatant (If more than 1.5 ml of bacterial culture is used, repeat this step). ★Use the remaining supernatant to resuspend the pellet.
	Fresh Blood/Frozen Blood ★Collect fresh blood/frozen blood in EDTA-NA_2 treated collection tubes (or other anticoagulant mixtures). ★Transfer up to 300 µl of fresh blood/frozen blood to a 1.5 ml microcentrifuge tube. If the blood sample is more than 300 µl (up to 1 ml), add the sample to a sterile 15 ml centrifuge tube (If using frozen blood samples proceed directly to Step 1 Lysis). ★**If using Fresh Blood samples**, add 3 x the sample volume of RBC Lysis Buffer and mix by inversion. Do not vortex. ★Incubate the tube for 10 minutes at room temperature. ★Centrifuge for 5 minutes at 3,000 x g and **remove the supernatant completely**. ★Add 100 µl of RBC Lysis Buffer to resuspend the cell pellet.

Step 1 Lysis	**Tissue** ★Add **350 µl of NR Buffer** to the sample in the mortar and grind the sample until it is completely dissolved. **If only Genomic DNA is required, add 2 µl of RNase A (10 mg/ml)**. ★Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
	Cultured Animal/Bacterial Cells ★Add **350 µl of NR Buffer** to the sample and mix completely. **If only Genomic DNA is required, add 2 µl of RNase A (10 mg/ml)**.
	Fresh Blood/Frozen Blood ★Add **350 µl of NR Buffer** to the fresh blood/frozen blood sample and mix completely. **If only Genomic DNA is required, add 2 µl of RNase A (10 mg/ml)**.
	Serum ★Add **350 µl of NR Buffer** to 100 µl of serum and mix completely. **If only Genomic DNA is required, add 2 µl of RNase A (10 mg/ml).**
	★Incubate Tissue, Cultured Cells, Bacteria, Fresh Blood and Serum samples at 60ºC for 10 minutes. When using frozen blood samples incubate, at 90ºC for 30 minutes. ★Incubate at 15-30ºC for 5 minutes. ★Microcentrifuge at 2-8ºC for 15 minutes.
Step 2 Isolation	★Transfer the supernatant to a new 1.5 ml microcentrifuge tube and add a 1/10 volume of NR Buffer and 600 µl of chloroform. ★Shake vigorously and then centrifuge at full speed for 10 minutes. ★Carefully remove the upper layer and transfer it to a new 1.5 ml microcentrifuge tube (repeat the Isolation Step until the interphase becomes clear).
Step 3 Nucleic Acid Precipitation	★Carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube containing 1 ml of Isopropanol. ★Gently invert the tube 3-5 times. ★Incubate at 15-30ºC for 10 minutes. ★Centrifuge at full speed for 15 minutes. ★Discard the supernatant and wash the pellet with 1 ml of 70% ethanol. ★Centrifuge at 2-8ºC at full speed for 5 minutes. ★**Completely discard the supernatant** and add 50-100 µl of RNase-free water to the 1.5 ml microcentrifuge tube. ★Incubate for 10 minutes at 60ºC to dissolve the pellet.

Yeast Protocol

Additional Requirements:

Microcentrifuge tubes, Absolute Ethanol for preparing 70% Ethanol in water, Chloroform, Isopropanol, Mortar and Pestle. sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol). Solutions (water and other solutions) should be treated with 0.1% DEPC.

Optional requirements:

If a larger sample volume is required, scale the NR Buffer proportionately. For RNA degradation, add RNase A (10 mg/ml) according to the protocol in the Step 1 Lysis. For DNA degradation, add 2 µl of DNase I (2 KU/ml), mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the final sample in the Nucleic Acid Precipitation Step. Let stand for 10 minutes at room temperature.

Sample Preparation Steps for Yeast Sample

1. Harvest fungus cells (up to 5 x 10^7) by centrifugation for 10 minutes at 5,000 x g.
2. Discard the supernatant and resuspend the pellet in 600 µl of sorbitol buffer.
3. Add 200 U of lyticase or zymolase. Incubate at 30ºC for 30 minutes.
4. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
5. Remove the supernatant and then follow the lysis step of Cultured Animal/Bacterial Cells protocol. Add 350 µl of NR Buffer to the sample and mix completely.

Troubleshooting

Problem	Possible Reasons/Solution
Incomplete Lysis	**Too much sample was used** ★Reduce sample volume or separate into multiple tubes and grind the sample completely
Low Yield	**Precipitate was formed at Step 3 Nucleic Acid Precipitation** ★Reduce the sample material. ★Increase incubation time following Isopropanol addition to improve total Nucleic Acid precipitation. ★Avoid RNase contamination.