

RNabsolute

For research use only

- Sample** : 50 mg of fresh tissue, cultured bacterial cells (up to 1×10^9),
cultured animal cells (up to 5×10^6), 300 μ l of blood or serum
- Operation time** : 60 minutes
- Efficiency** : High yield RNA ideal for Reverse Transcription Polymerase Chain
Reaction (RT-PCR) and Northern Blotting

Geneaid



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Introduction

RNabsolute provides an efficient 3 step method to isolate total RNA from tissue, cultured animal and bacterial cells, blood and serum. This unique reagent system ensures total RNA with high yield and good quality from samples of unlimited size. If a larger sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user friendly but also highly versatile. DNA phenol extraction is not required and the entire procedure can be completed in 60 minutes. The total RNA is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Quality Control

The quality of RNabsolute is tested on a lot-to-lot basis by isolating total RNA from 50 mg tissue samples. A minimum of 20 μ g of total RNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	RAR004	RAR100
RNabsolute*	4 ml	100 ml
RNabsolute 2*	500 μ l	15 ml

*If the reagents contain sediment, incubate at 65°C for 10 minutes to dissolve.

Order Information

Product Name	Reactions	Cat. No.
Reagent Genomic DNA Kit	100/1000 rxns	GE100/01K
DNabsolute	100 rxns	NR100
Plant DNabsolute	100 rxns	GR100
RNabsolute	100 rxns	RAR100
Plant RNabsolute	100 rxns	RPR100
96-Well DNabsolute	4/10 x 96 rxns	NRP04/10
96-Well Plant DNabsolute	4/10 x 96 rxns	GRP04/10

Caution

The components contain irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Additional requirements

mortar and pestle, microcentrifuge tubes (RNase free), RNase-free H₂O, β -mercaptoethanol, chloroform, absolute EtOH for preparing 70% EtOH in H₂O (RNase free), isopropanol

Optional requirements

- If a larger sample volume is required, scale the RNabsolute proportionately.
- For complete DNA Degradation, add 2 μ l of DNase I (2 KU/ml), mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 50 μ g/ml BSA at 25°C} to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.
- RBC Lysis Buffer

RNabsolute Protocol

Sample Preparation	<p>Tissue</p> <ul style="list-style-type: none"> ● Cut off 50 mg of fresh tissue. ● Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle. <p>Cultured Animal and Bacterial Cells</p> <ul style="list-style-type: none"> ● Transfer cultured animal cells (up to 5×10^6) or bacterial culture (up to 1×10^9) to a 1.5 ml microcentrifuge tube. ● Centrifuge at 14-16,000 x g for 1 minute and remove the supernatant completely (If more than 1.5 ml of bacterial culture is used, repeat this step). ● Use 20 μl of RNase-free ddH₂O to re-suspend the pellet. <p>Fresh Blood/Frozen Blood</p> <ul style="list-style-type: none"> ● Collect blood in EDTA-NA₂ treated collection tubes (or other anticoagulant mixtures). ● Transfer up to 300 μl of 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 3X 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	<p>Tissue</p> <ul style="list-style-type: none"> ● Add 500 μl of RNabsolute and 8 μl of β-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved. ● Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. <p>Cultured Animal and Bacterial Cells/Fresh Blood/Frozen Blood</p> <ul style="list-style-type: none"> ● Add 500 μl of RNabsolute and 8 μl of β-mercaptoethanol to the sample and mix completely. <p>Serum</p> <ul style="list-style-type: none"> ● Transfer 100 μl of serum to a 1.5 ml microcentrifuge tube. ● Add 500 μl of RNabsolute and 8 μl of β-mercaptoethanol and mix completely. <hr/> <ul style="list-style-type: none"> ● Incubate Tissue/Cultured Animal and Bacterial Cells/Fresh Blood/Serum samples at 60°C for 10 minutes. When using Frozen Blood samples incubate at 90°C for 30 minutes. ● Incubate at 15-30°C for 5 minutes. <p>For Frozen Blood or Tissue (for all other samples please proceed directly to step 2)</p> <ul style="list-style-type: none"> ● Centrifuge at 14-16,000 x g at 2-8°C for 15 minutes and transfer the supernatant to a new 1.5 ml microcentrifuge tube.
Step 2 Phase Separation	<ul style="list-style-type: none"> ● Add a 1/10 volume of RNabsolute 2 and 500 μl of chloroform to either the mixture or supernatant from Step 1 (depending on sample type). ● Shake vigorously and then centrifuge at 14-16,000 x g for 5 minutes. ● Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube. <p>Repeat the Phase Separation Step until the interphase becomes clear by adding only 500 μl of chloroform (per repetition) to the upper phase in the new 1.5 ml microcentrifuge tube. Once the interphase becomes clear, transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.</p> <p>NOTE: The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.</p>
Step 3 RNA Precipitation	<ul style="list-style-type: none"> ● Add 500 μl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 2. ● Gently invert the tube 3-5 times. ● Incubate on ice for 10 minutes. ● Centrifuge at 14-16,000 x g for 15 minutes. ● Discard the supernatant and wash the pellet with 1 ml of 70% EtOH. ● Centrifuge at 14-16,000 x g at 2-8°C for 5 minutes. ● Completely discard the supernatant and add 50-100 μl of RNase-free H₂O to the 1.5 ml microcentrifuge tube. ● Incubate for 10 minutes at 60°C to dissolve the pellet.

Troubleshooting

Problem	Possible Reasons/Solution
Incomplete Lysis	<p>Too much sample was used</p> <ul style="list-style-type: none"> ● Reduce sample volume or separate into multiple tubes and grind the sample completely
Low Yield	<p>Precipitate was formed at Step 3 RNA Precipitation</p> <ul style="list-style-type: none"> ● Reduce the sample material ● Increase incubation time following Isopropanol addition to improve total RNA precipitation ● Avoid RNase contamination