DNabsolute

For research use only

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Sample : 50 mg of fresh tissue, cultured bacterial cells (up to 1 x 10^9), cultured animal cells (up to 5 x 10^6), 300 µl of blood or serum

Operation time : within 60 minutes

Efficiency : High yield DNA ideal for Polymerase Chain Reaction (PCR)

Introduction

DNabsolute provides an easy 3 step method to isolate high yields of total DNA (from tissue, cultured animal and bacterial cells, blood and serum) that is ideal for use in Polymerase Chain Reaction. This unique reagent ensures total DNA with high yield and good quality from samples of unlimited size. If a large 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 total DNA is ready for use in PCR, RT-PCR, Southern Blotting, Northern Blotting, Mapping and RFLP.

Quality Control

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

Kit Contents

Name	NR004	NR100
DNabsolute*	4 ml	100 ml
RNase A (50 mg/ml)** (store at -20°C before use)	Provided by user	50 µl

*If DNabsolute contains sediment, incubate at 65°C for 10 minutes to dissolve.

**Do not add RNase A to DNabsolute prior to use.

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, absolute EtOH for preparing 70% EtOH in H_2O, chloroform, isopropanol, TE or ddH_2O

Optional requirements

- If a larger sample volume is required, scale DNabsolute proportionately
- RBC Lysis Buffer

DNabsolute Protocol

Sample Preparation	**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 bacterial culture (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube. • Centrifuge at 14-16,000 x g for 1 minute and pour off the majority of the supernatant (If more than 1.5 ml of bacterial culture is used, repeat this step). • Use the remaining supernatant to re-suspend the pellet. **Fresh Blood/Frozen Blood** • Collect blood in EDTA-NA_2 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 re-suspend the cell pellet.
Step 1 Lysis	**Tissue** • Add **350 µl of DNabsolute** and **0.5 µl of RNase A (50 mg/ml)** 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. **Cultured Animal and Bacterial Cells/Fresh Blood/Frozen Blood** • Add **350 µl of DNabsolute** and **0.5 µl of RNase A (50 mg/ml)** to the sample and mix completely. **Serum** • Transfer 100 µl of serum to a 1.5 ml microcentrifuge tube. • Add **350 µl of DNabsolute** and **0.5 µl of RNase A (50 mg/ml)** and mix completely. --- • Incubate Tissue/Cultured Animal and Bacterial Cells/Fresh Blood/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. **For Frozen Blood or Tissue (for all other samples proceed directly to Step 2)** • Centrifuge at 14-16,000 x g at 2-8ºC for 15 minutes and transfer the supernatant to a new 1.5 ml microcentrifuge tube.
Step 2 Phase Separation	• Add a **1/10 volume of DNabsolute** and 600 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 10 minutes. • Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube. **Repeat the Phase Separation Step until the interphase becomes clear by adding only 600 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.** NOTE: The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.
Step 3 DNA Precipitation	• Add 1 ml of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 2. • Mix the sample by gently inverting the tube 3-5 times. • Incubate at 15-30ºC 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 2-8ºC at 14-16,000 x g for 5 minutes. • **Completely discard the supernatant** and add 50-100 µl of TE or ddH_2O to the 1.5 ml microcentrifuge tube. • Incubate for 10 minutes at 60ºC to dissolve the pellet.

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 DNA Precipitation** • Reduce the sample material • Increase incubation time following isopropanol addition to improve total DNA precipitation