Extract Reagent (Genomic DNA Isolation Reagent)

Cat. No.: SN001-0100 Size: 100 ml
Cat. No.: SN001-0004 Size: 4 ml
Sample: 50 mg of fresh tissue,
5 x 10^6celture animal cells
1X 10^9 bacterial cells
Format: Reagent form
Operation time: 90 minutes
Elution volume: 50~100 µ

Description

Extract Reagent (Genomic DNA Isolation Reagent) provides an easy 3-step method to isolate high yields of total DNA (from tissue, cultured animal and bacterial cells, blood and serum). This unique reagent ensures total DNA with a 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. The DNA phenol extraction is not required and the entire procedure can be completed in 90 minutes.

Features

- Fast procedure and delivering high-quality genomic DNA
- Ready-to-use DNA for high performance in any downstream application
- Consistent DNA yields from a small amount of the starting material
- Time flexibility
- Ease of DNA extraction technique or method
- Expense reduction

Applications

- Quantity of DNA needed
- Purity of DNA required
- Molecular weight and size of DNA
- Downstream applications of DNA

Kit Content

Content	SN001-0100	SN001-0004
Extract Reagent (Genomic DNA Isolation Reagent)	100 ml	2 ml X 2 vials

Quality Control

The quality of the Extract Reagent (Genomic DNA Isolation Reagent) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Homogenizer
- RNase A (50 mg/ml)
- Chloroform
- Isopropanol
- 70% ethanol
- Water Bath / Dry Bath
- Microcentrifuge tubes
- TE (Tris-EDTA, pH8.0) or ddH_2O

Buffer Preparation

TE Buffer (Tris-EDTA, pH8.0) : 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA

Extract Reagent (Genomic DNA Isolation Reagent) Protocol

Sample Preparation

Tissue

1. Prepare 50 mg of the fresh tissue, approximately.
2. Grind the sample in the liquid nitrogen to a fine powder using a homogenizer.

Cultured Animal/Bacterial Cells

1. 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.
2. 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).
3. Use the remaining supernatant to re-suspend the pellet.

Fresh Blood/Frozen Blood

1. Collect blood in EDTA-NA2 treated collection tubes (or other anticoagulant mixtures).
2. 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.

Step 1 Lysis

Tissue

1. Add 350 µl of Extract Reagent (Genomic DNA Isolation Reagent) and 0.5 µl of RNase A (50 mg/ml) to the sample in the homogenizer and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate tissue samples at 60ºC for 10 minutes.
4. Incubate at 15~30ºC for 5 minutes.
5. 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.

Cultured Animal and Bacterial Cells/Fresh blood/Frozen Blood

1. Add 350 µl of Extract Reagent (Genomic DNA Isolation Reagent) and 0.5 µl of RNase A (50 mg/ml) to the sample and mix completely.
2. Incubate cultured animal and bacterial cells/ fresh blood samples at 60ºC for 10 minutes. When using the frozen blood samples, incubate at 90ºC for 30 minutes.
3. Incubate at 15~30ºC for 5 minutes.

For frozen blood, 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.

Serum

1. Transfer 100 µl of the serum to a 1.5 ml microcentrifuge tube.
2. Add 350 µl of the Extract Reagent (Genomic DNA Isolation Reagent) and 0.5 µl of the RNase A (50 mg/ml) and mix completely.
3. Incubate serum samples at 60ºC for 10 minutes.
4. Incubate at 15~30ºC for 5 minutes.

Step 2 Phase Separation

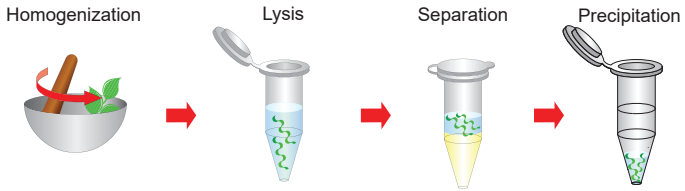
1. Add a 1/10 volume of the Extract Reagent (Genomic DNA Isolation Reagent) and 600 µl of the chloroform to the supernatant from Step 1. Shake vigorously and then centrifuge at 14~16,000 x g for 10 minutes.
2. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.

3. Repeat the Phase Separation Step until the interphase becomes clear, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

NOTE: The number of repetitions is dependent on the sample type; e.g. dense tissue samples may require a higher number of repeats.

Step 3 DNA Precipitation

1. Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from the Step 2.
2. Mix the sample by inverting gently and letting it stand for 5 minutes at the room temperature (The DNA precipitation can be increased with extended standing time).
3. Centrifuge at 14~16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of 70% ethanol.
5. Centrifuge at 14~16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50~100 µl of TE buffer (not provided) or ddH_2O.
7. Incubate for 10 minutes at 60oC to dissolve the pellet.



Troubleshooting

Problem	Cause	Solution
Difficult to dissolve	Incomplete removal of EtOH	Remove EtOH in the hood briefly.
RNA containment	Incomplete removal of RNA	RNase A treatment.
Low yields of genomic DNA	Incomplete lysis and homogenization	Complete lysis and homogenization. Use the appropriate method for the lysate preparation based on the amount of the starting materials. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Extract Reagent to achieve the optimal lysis.
	Presence of EtOH	Remove EtOH in the hood briefly.
	Incorrect elution conditions	Add TE Buffer or RNase-free H_2O (50~100 µl) and incubate for 10 min at 60°C.
	Incorrect separation phase	Repeat the Phase Separation Phase until the interphase becomes clear, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.
A260/280 ratio is below	Incomplete removal of the Protein	Increase the amount of time the proteins are allowed to precipitate

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM201-0100	Hot Start SUPERMIX	100 Reactions
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml
SD010-R500	1 Kb DNA Ladder RTU	500 µl
SD013-R500	XLarge DNA Ladder RTU	500 µl
SN025-1000	2.5 mM dNTP Mix	1 ml
SN100-1000	25 mM dNTP Mix	1 ml

Caution

- Check the buffer before use for salt precipitation.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.