GD Reagent
(Genomic DNA Isolation Reagent)

Cat No. PDR01-0100/PDR01-0100S
Size: 100 ml/4 ml

Description

GD 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 proportion-ately, 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. The extracted total DNA is ready for use in PCR, Real-time PCR, Southern Blotting, Mapping and RFLP.

Background on Genomic DNA Isolation and Purification

The goal of the genomic DNA isolation depends on the type(s) of DNA application after isolation. The DNA's purity, source, quantity, and quality are all key issues that need to be addressed prior to the genomic DNA extraction. A whole host of different methods, technologies and kits are available now to researchers to isolate the genomic DNA from cells. The DNA is isolated from proteins by several methods, including the digestion of proteins by the enzyme proteinase K. The proteins are removed subsequently by salting-out, organic extraction, or binding of the DNA to a solid-phase support (such as an anion-exchange column or silica technology). The DNA is finally recovered by ethanol precipitation or isopropanol precipitation.

Generally, all methods involve the disruption and lysis of cells. This is followed sometimes by the removal of RNA by RNAses, salt or other methods. The DNA is isolated from the proteins by several methods, including the digestion of proteins by the enzyme proteinase K. The proteins are removed subsequently by salting-out, organic extraction, or binding of the DNA to a solid-phase support (such as an anion-exchange column or silica technology). The DNA is finally recovered by ethanol precipitation or isopropanol precipitation.

The separation of the DNA from cells and cellular components can be divided into four stages:
Cell disruption
Lysis of Cell
Removal of Proteins and Contaminants
Recovery of DNA

Feature

- Fast procedure delivering high-quality genomic DNA
- Ready-to-use DNA for high performance in any downstream application
- Consistent DNA yields from small amounts of the starting material
- Provide sufficient reagents and 3 steps to treat samples from 300 µl to 1.5 ml
- Time flexibility
- Ease of DNA extraction technique or method
- Expense reduction

Application

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

Required Volume of Lysis Buffer

Cell	Required Sample	Required Lysis Buffer
Tissue	50 mg	350 ul
Culture Cell	1.5 mL	350 ul
Fresh Blood / Frozen Blood	300 µl	350 ul

Required Materials

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

Buffer Preparation

- TE Buffer (Tris-EDTA, pH8.0): 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA

GD Genomic DNA Isolation Protocol

Sample Preparation

Tissue

1. Cut off 50 mg of the fresh tissue.
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-NA_2 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 GD 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.

Cultured Animal and Bacterial Cells/Fresh blood/Frozen Blood

Add 350 µl of GD Reagent and 0.5 µl of RNase A (50 mg/ml) to the sample and mix completely.

Serum

1. Transfer 100 µl of the serum to a 1.5 ml microcentrifuge tube.
2. Add 350 µl of the GD Reagent and 0.5 µl of the RNase A (50 mg/ml) and mix completely.
3. 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.
4. 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.

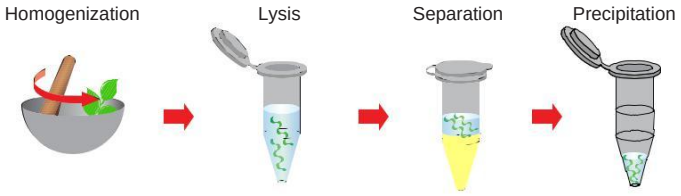
Step 2 Phase Separation

1. Add a 1/10 volume of the GD 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, 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 tempera ture (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% EtOH.
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
	Overdry	Don't overdry the pellet
RNA containment	Incomplete removal of RNA	RNase A treatment
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove EtOH in the hood briefly
	Presence of salt in purified RNA	Use 70% EtOH in the correct order
Low yields of gDNA	Incomplete lysis and homogenization	Complete the lysis and homogenization (tissue) thoroughly Use the appropriate method for lysate preparation based on the amount of starting materials Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the GD Reagent to achieve the optimal lysis
	Presence of EtOH	Remove EtOH in the hood briefly
	Incorrect elution conditions	Add RNase-free H_2O (50~100µl) or TE Buffer, and incubate for 10 min at 60ºC
	The quality of the starting material may not be optimal.	Increase the amount of the starting material, and scale up the volumes of the reagents accordingly
	Incorrect DNA precipitation	Increase the amount of time for the DNA Precipitation Step.
A260/280 ratio is below	Incomplete removal of the Protein	Increase the amount of time the proteins are allowed to precipitate

Caution

Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
During the operation, always wear a lab coat, disposable gloves, and protective equipment.
Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.