DG reagent (Genomic DNA Isolation Kit)

(Tissue, Cultured Animal and Bacterial Cells, Blood and Serum)

For research use only

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Introduction

DG 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 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 entire procedure can be completed in 90 minutes. The extracted total DNA is ready for use in Southern Blotting, PCR, Real-time PCR, Mapping and RFLP.

Kit Content

Catalog No.	DG0004	DG0100	DG0500
DG Reagent	4 ml	100 ml	500 ml

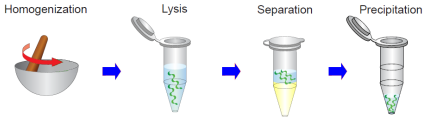
Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the FB DG Reagent (Plant Genomic DNA Isolation Kit) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

∗mortar and pestle ∗microcentrifuge tubes ∗70% EtOH ∗chloroform ∗isopropanol ∗RNase A (50 mg/ml) ∗TE or ddH_2O

DG reagent (Genomic DNA Isolation Kit) 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.

Serum

- Transfer 100 µl of serum to a 1.5 ml microcentrifuge tube.

Step 1 Lysis

Tissue

- Add **350 µl of DG reagent** 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.
- Incubate at 60ºC for 15 minutes.
- 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

- Add **350 µl of DG reagent** and **0.5 µl of RNase A (50 mg/ml)** to the sample and mix completely.
- Incubate at 60ºC for 10 minutes.

Frozen Blood

- Add **350 µl of DG reagent** and **0.5 µl of RNase A (50 mg/ml)** to the sample and mix completely.
- Incubate at 90ºC for 30 minutes then incubate at 15-30ºC for 5 minutes.
- 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

- Add **350 µl of DG reagent** and **0.5 µl of RNase A (50 mg/ml)** and mix completely.
- Incubate at 60ºC for 10 minutes.

Step 2 Phase Separation

- Add a **1/10 volume of DG reagent** and 600 µl of chloroform to the supernatant from Step 1.
- 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 then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube. (**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 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step2.
- Mix the sample by inverting gently and let stand for 5 minutes at room temperature (DNA precipitation can be increased with extended standing time).
- 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 for 5 minutes. **Completely discard the supernatant** and re-suspend the pellets in 50-100 µl of TE buffer or ddH_2O.
- Incubate for 10 minutes at 60ºC to dissolve the pellet.

Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	For each different sample type, use only the required range or amount of starting materials to prepare the lysates.
		For tissues, cut the tissue into smaller pieces and ensure that the tissue is completely immersed in the Lysis step to obtain optimal lysis.
DNA degradation	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample. Use a new sample for DNA isolation. Perform the extraction using fresh materials whenever possible.
	Inappropriate sample storage conditions	Store mammalian tissues at -80°C and bacteria at -20°C until use. The whole blood can be stored at 4°C for no longer than 1-2 days.
	DNase contaminantion	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents).
Inhibition of downstream enzymatic reactions	Purified DNA containing residual ethanol	Remove EtOH in the hood briefly.
Presence of RNA	RNA contamination	Perform RNase A digestion step during the Lysis Step.