Genomic DNA Isolation Reagent Kit (Blood/Cultured Cell/Tissue) www.fairbiotech.com

For research use only

Sample: whole blood, buffy coat, mammalian cells, fungus cells, bacterial cells and tissue

Introduction

The Genomic DNA Isolation Reagent Kit is a reagent system kit. The kit was designed specifically for genomic DNA isolation from whole blood, frozen blood, buffy coat, cultured animal/bacterial cells, fungus and tissue. This unique reagent system ensures genomic DNA with high yield and good quality from samples The entire procedure can be completed in 1 hour without phenol/chloroform extraction. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

Kit Contents

Catalog No.	BB0004	BB0100	BB0200	BB0300
BR Buffer	4 ml	100 ml	100 ml×2	100 ml×3
BC Buffer	1.5 ml	35 ml	65 ml	95 ml
BP Buffer	0.5 ml	12 ml	25 ml	35 ml

Quality Control

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

Additional requirements

＊microcentrifuge tubes ＊Isopropanol ＊RNase A (10 mg/ml) ＊For Tissue sample: Proteinase K(10 mg/ml), Micropestle

＊For Gram-positive bacterial samples: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)

＊For Fungual samples: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

NOTE

★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

Protocol

Step 1 Sample Cells Harvesting

Tissue

- Transfer 30 mg animal tissue to a sterile 1.5 ml microcentrifuge tube.
- Use a micropestle to grind the tissue a few times.

Fresh whole Blood or Buffy Coat

- Collect blood in EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
- Transfer up to **300 µl of blood** or **200 µl of buffy coat** to a sterile1.5 ml microcentrifuge tube.
- Add **900 µl of BR Buffer** and mix by inversion.
- Incubate the tube at room temperature for 10 minutes (invert twice during incubation).
- Centrifuge for 5 minutes at 4,000 x g. **Remove the supernatant completely** and resuspend the cells in **50 µl of BR Buffer** by pipetting the pellet up and down.

Cultured Mammalian Cells

- Transfer cultured mammalian cells (up to 10^7) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 6,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **50 µl of BR Buffer** by pipetting the pellet up and down.

Gram-Negative Bacterial Cells

- Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 12,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **50 µl of BR Buffer** by pipetting the pellet up and down.

Gram-Postive Bacterial Cells

- Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 12,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **100 µl of lysozyme Buffer** by pipetting the pellet up and down. Incubate at room temperature for 20 minutes.

Fungus Cells

- Transfer fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 6,000 x g for 5 minute. **Remove the supernatant completely** and resuspend the cells in **600 µl of sorbitol Buffer** by pipetting the pellet up and down.
- Add **200 U of lyticase or zymolase**. Incubate at 30ºC for 30 minutes.
- Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. **Remove the supernatant completely** and hresuspend the cells in **50 µl of BR Buffer** by pipetting the pellet up and down.

Step 2 Lysis

Tissue

- Add **300 µl of BC Buffer** and **20 µl of Proteinase K(10mg/ml)** to the tube from Step 1 and continually homogenize the sample tissue with grinding.
- Incubate at 70˚C for 30 minutes and invert the tube every 5 minutes. If the lysate has not become totally clear at the 30 minute mark, use a micropestle to grind the remaining pellet and place the sample back at 70˚C until it is clear.

Blood/Cultured Cell

- Add **300 µl of BC Buffer** to the resuspended cells from Step 1 and mix by vortex.
- Incubate at 70ºC for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step:

RNA Degradation (If RNA-free genomic DNA is required, perform this optional step.)

- Add 5 µl of RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal

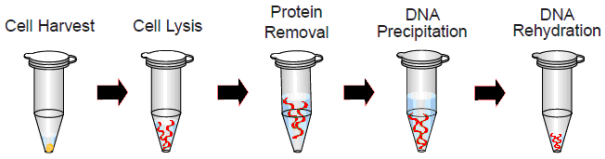
- Add **100 µl of BP Buffer** to the sample lysate and vortex immediately for 10 seconds.
- Incubate on ice for 5 minutes. Centrifuge at 14-16,000 x g for 3 minutes.
- Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation

- Add 300 µl of Isopropanol to the sample from step 3 and mix well by inverting 20 times.
- Centrifuge at 14-16,000 x g for 5 minutes.
- Discard the supernatant carefully and add 300 µl of 70% ethanol to wash the pellet.
- Centrifuge at 14-16,000 x g for 3 minutes.
- Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration

- Add 50-100 µl of TE buffer or dd water and incubate at 70ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.



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.
		Be sure to add Proteinase K during lysis.
		Increase the digestion time or amount of Proteinase K used for lysis.
		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.