Genomic DNA Isolation Dual Kit (Blood/Cultured Cell)

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For research use only

Sample: up to 300 µl of whole blood, 200 µl of buffy coat, 10^7 mammalian cells, 5×10^7 fungus cells and 10^9 bacterial cells
Yield : up to 50 µg

Introduction

The Genomic DNA Isolation Dual Kit (Blood/Cultured Cell) combines a reagent system with a spin column system. The kit was designed specifically for genomic DNA isolation from whole blood, frozen blood, buffy coat, cultured animal/bacterial cells and fungus. This unique reagent system ensures genomic DNA with high yield and good quality from the samples while the spin column system purify or concentrate genomic DNA products which have been previously isolated using Reagents. 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.	DB0004	DB0100	DB0200	DB0300
RL Buffer	4 ml	100 ml	100 ml×2	100 ml×3
CL Buffer	1.5 ml	35 ml	65 ml	95 ml
PO Buffer	0.5 ml	12 ml	25 ml	35 ml
BD Buffer	2 ml	45 ml	85 ml	125 ml
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300µl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml×2 (100ml)×2
EL Buffer	1 ml	10 ml	20 ml	30 ml
DB Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

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

Additional requirements

∗ microcentrifuge tubes ∗ Isopropanol ∗ absolute ethanol
∗ RNase A (10 mg/ml)

∗ For Gram-positive bacteria sample:
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 Fungus sample:
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

★ Add ethanol (96–100%) to Buffer W2, **shaking before use** (see bottle label for volume).
★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
★ Buffers W1 contain irritants. Wear gloves when handling these buffers.

Reagent System Protocol

Step 1 Sample Cells Harvesting

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 RL 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 RL 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 RL Buffer** by pipetting the pellet.

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 RL Buffer** by pipetting the pellet.

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 resuspend the cells in **50 µl of RL Buffer** by pipetting the pellet up and down.

Step 2 Lysis

- Add **300 µl of CL Buffer** to the resuspended cells from Step 1 and mix by vortex.
- Incubate at 60ºC 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.

Switch Step

- If purer DNA is required, skip Step 3 – 5, and proceed with Step 1 of the Column-Based DNA Purification protocol on the next page.

Step 3 Protein Removal

- Add **100 µl of PO 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 and add 300 µl of 70% ethanol to wash the pellet.
- Centrifuge at 14-16,000 x g for 3 minutes.
- Discard the supernatant and air-dry the pellet for 10 minutes.

Step 5 DNA Rehydration

- Add 50-100 µl of **EL Buffer** and incubate at 60ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to facilitate DNA rehydration.

Column System (DNA Purification) Protocol

Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use.

Pre-heat the Elution Buffer to 60ºC prior to use.

Step 1 Protein Removal

- Add **400 µl of BD Buffer** to the sample from Reagent System Protocol **Step 2 Lysis** and shake vigorously.
- Centrifuge at 14-16,000 x g for 1 minutes. (Don`t over 1 minute)

Step 2 DNA Binding

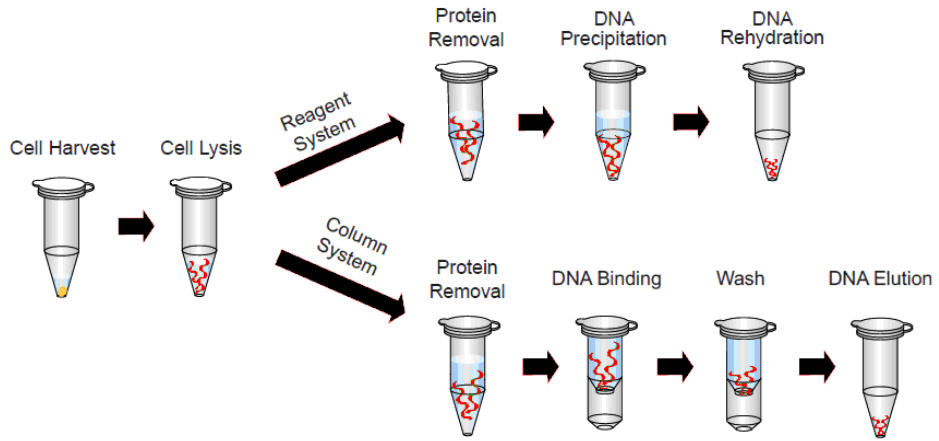
- Place a **DB Column** in a **2 ml Collection Tube**.
- Transfer the clear supernatant completely from the previous step to the **DB Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the **DB Column** back in the **2 ml Collection Tube**.

Step 4 Wash

- Add 400 µl of **W1 Buffer** into the **DB Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **DB Column** back into the same **Collection tube**.
- Add 600 µl of **W2 Buffer** (Ethanol added) into the **DB Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **DB Column** back into the same **Collection tube.**
- Centrifuge at 14,000 x g again for 2 minutes to remove residual **W2 Buffer**.

Step 5 DNA Elution

- Transfer the dried **DB Column** to a new 1.5 ml microcentrifuge tube.
- Add **50-200 µl of Pre-Heated EL Buffer** or TE into the center of the column matrix.
- Let stand at 60ºC for 5 minutes.
- Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	Decrease the sample amount prior to use.
	Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the W2 Buffer prior to use.
	Buffer EL pH is too low	Check the pH.
	Buffer EL not pre-heated at 60°C	Pre-heat the Elution Buffer to 60°C prior to use.
DNA degradation	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample. Use a new sample for the DNA isolation. Perform the extraction of the fresh material when possible.
	Inappropriate sample storage conditions	Store bacteria at -20°C until use. Whole blood can be stored at 4°C for no longer than 3~5 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	If the residual solution is seen in the purification column after washing the column with the Buffer W2, empty the collection tube and re-spin the column for an additional 1 min. at the maximum speed (≥12000 x g).
	Purified DNA contains residual salt	Use the Wash Buffers in their correct order. Always wash the purification column with Buffer W1 first and then proceed to the wash step with Buffer W2.