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Genomic DNA Extraction Kit (Whole Blood / Buffy coat)

For research use only

Sample: up to 200 µl of whole blood, buffy coat, plasma, serum, body fluids or 5×10^6 lymphocytes

Yield　: up to 50 µg

Introduction

This BioDiamond Genomic DNA Extraction Kit (Cultured Cell/Blood) was designed specifically for genomic DNA isolation from whole blood, frozen blood and buffy coat. Its unique buffer system ensures genomic DNA with high yield and good quality from samples while the spin column purifies and concentrates genomic DNA products previously isolated with the buffer system. The entire procedure can be completed in 1 hour without phenol/chloroform extraction needs. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

Kit Contents

Catalog No.	DMGWB100	DMGWB300
CK Buffer	25 ml	65 ml
W1 Buffer	45 ml	125 ml
W2 Buffer (Add Ethanol)	15 ml (60 ml)	25 ml × 2 (100 ml × 2)
EL Buffer	10 ml	30 ml
CC Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs
Proteinase K (Add ddH_2O)	40 mg (2 ml)	120 mg (6 ml)

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the BioDiamond Genomic DNA Extraction Kit (Cultured cell/ Blood) tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

＊microcentrifuge tubes ＊ absolute ethanol

＊RNase A (10 mg/ml)

NOTE

★ Add ethanol (96–100%) to Buffer W2, **shake before use** (see bottle label for volume).

★ Add ddH_2O to the proteinase K, vortex to dissolve and **shake 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.

Protocol

Step 1 Sample Cells Harvesting

- Collect blood in EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
- Transfer up to **200 µl of blood** or **200 µl of buffy coat** to a sterile1.5 ml microcentrifuge tube.

Step 2 Lysis

- Add **20 µl of proteinase K and 200 µl of CK Buffer** to the sample from Step 1 and mix by pulse-vortexing for 15 s.
- Incubate at 55ºC for 10 minutes. During incubation, invert the tube every 3 minutes.

#Pre-heat the Elution Buffer to 60ºC for Step 6 DNA Elution.

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 DNA Binding

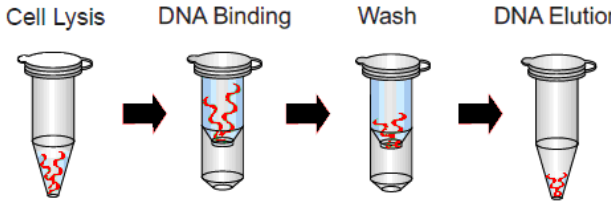
- Add **350 µl of ethanol (96-100%)** to the sample from Step 2 and mix by pulse-vortexing for 15 s.
- Place a **CC Column** in a **2 ml Collection Tube**.
- **Transfer the mixture completely** to the **CC Column**.
- Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **CC Column** back in the **2 ml Collection Tube**.

Step 4 Wash

- Add **400 µl of W1 Buffer** into the **CC Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **CC Column** back into the same **Collection tube**.
- Add **600 µl of W2 Buffer** (Ethanol added) into the **CC Column**. Centrifuge at 14,000 x g for 30 seconds.
- Discard the flow-through and place the **CC 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 **CC 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 55ºC for 3 minutes.
- Centrifuge for 2 minutes at 14,000 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 to 55°C	Pre-heat the Elution Buffer to 55°C prior to use.
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	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 residual solution is seen in the purification column after washing the column with 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.