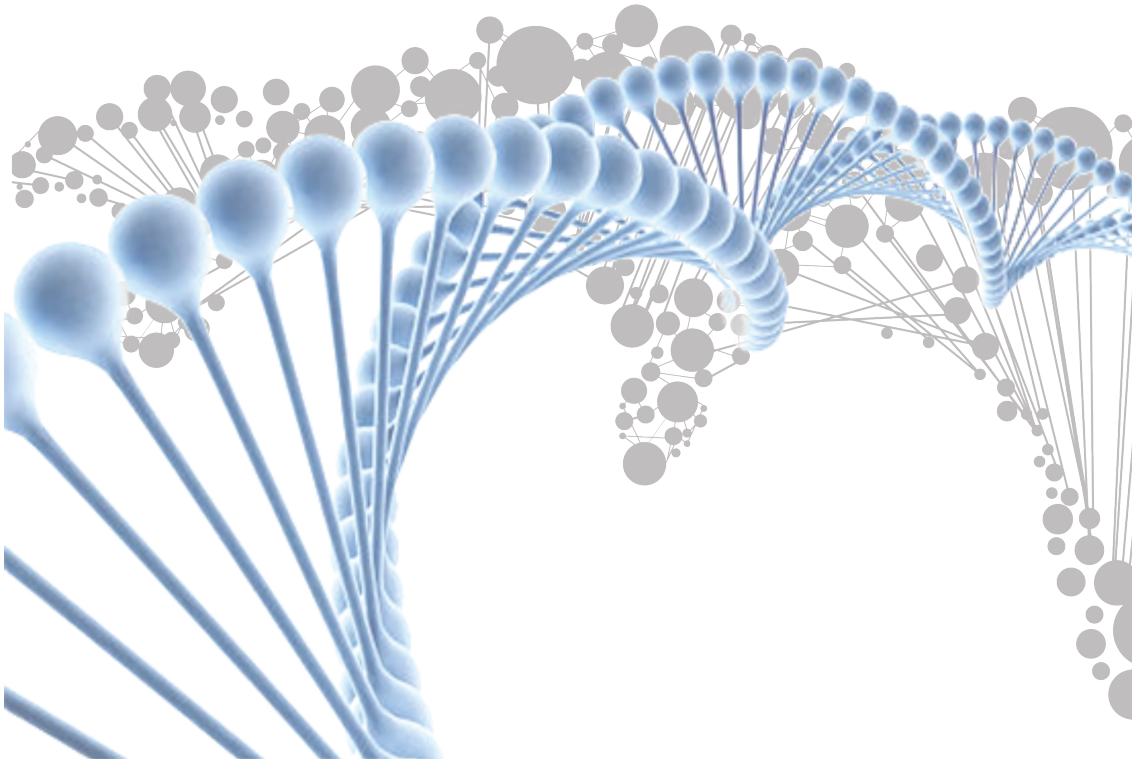
MGK-C0100 & MGK-C0300

Genomic DNA Isolation --Cell/ Blood



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Genomic DNA Isolation Kit (Cell/ Blood)

Storage: Stored at room temperature (15-25°C).
Format: spin column **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.

Introduction:

This spin-column based Genomic DNA Isolation Kit (Cultured Cell/Blood) was designed specifically for genomic DNA isolation from whole blood, frozen blood, buffy coat, cultured animal/bacterial cells and fungal cells. 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.

About the kits:

Cat. No.	MGK-C0100	MGK-C0300
SD Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs
C1 Buffer	100 ml	100 ml x3
C2 Buffer	35 ml	95 ml
C3 Buffer	45 ml	125 ml
WB1 Buffer	45 ml	125 ml
WB2 Buffer	15 ml	25 ml x2
Elution Buffer	10 ml	30 ml

Additional requirements:

* Ethanol (96~100%)
* RNase A (10 mg/ml)
* For **Gram(+) bacteria 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 Fungus samples**: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35mM ß-mercaptoethanol)

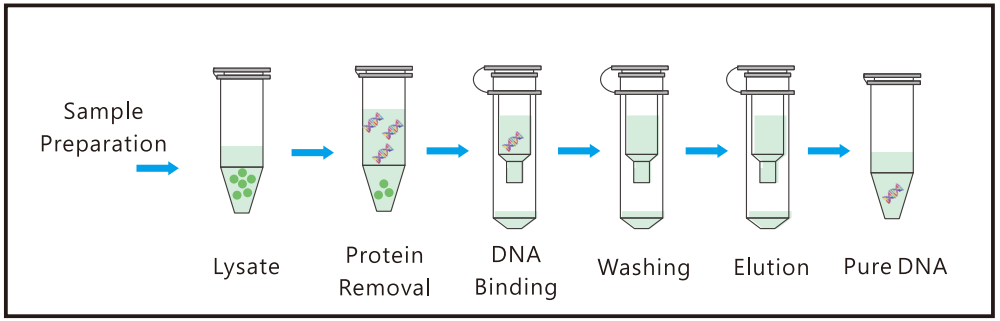
NOTE:

1. Add ethanol (96–100%) to Buffer WB2 before use (see bottle label for volume).
2. If necessary, re-dissolve any precipitate by warming to 37°C.
3. Buffers W1 contain irritants. Wear gloves when handling these buffers.

Related product and Ordering information:

CAT NO:	Product	Quantity
MGK-T0100	EBL Genomic DNA Isolation Kit (Tissue)	100 rxn.
MGK-T0300	EBL Genomic DNA Isolation Kit (Tissue)	300 rxn.
MGK-C0100	EBL Genomic DNA Isolation Kit (Cell/ Blood)	100 rxn.
MGK-C0300	EBL Genomic DNA Isolation Kit (Cell/ Blood)	300 rxn.
MGK-P0100	EBL Genomic DNA Isolation Kit (Plant)	100 rxn.
MGK-P0300	EBL Genomic DNA Isolation Kit (Plant)	300 rxn.
MPD-01300	EBL Plasmid Miniprep DNA Kit	300 rxn.
MPD-02025	EBL 200PLUS Plasmid Midiprep DNA Kit	25 rxn
MPD-03020	EBL 800PLUS Plasmid Maxiprep DNA Kit	20 rxn
FBRE100	Blood/ cell Total RNA Isolation kit	100 rxn.
FBRT100	Tissue Total RNA Isolation kit	100 rxn.
FBRP	Plant Total RNA Isolation kit	100 rxn.

Procedure:



Protocol:

Step 1. Sample Preparation

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 C1 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 C1 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 C1 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 C1 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 30°C 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 C1 Buffer by pipetting the pellet up and down.

Step 2. Lysis

> Add 300 µl of C2 Buffer to the resuspended cells from Step 1 and mix by vortex.
> Incubate at 60°C for 10 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes.

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

Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step.)

> Add 5 µl of RNase A (50 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 C3 Buffer to the sample from Step 2 and shake vigorously.
> Centrifuge at 14,000 x g for 1 minute. **(Do not go over 1 minute)**

Step 4. DNA Binding

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

Step 5. Wash

> Add 400 µl of WB1 Buffer into the SD Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SD Column back into the same Collection tube.
> Add 600 µl of WB2 Buffer (Ethanol added) into the SD Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SD Column back into the same Collection tube.
> Centrifuge at 14,000 x g again for 2 minutes to remove residual WB2 Buffer.

Step 6. DNA Elution

> Transfer the dried SD Column to a new 1.5 ml microcentrifuge tube.
> Add 50-200 µl of Pre-Heated Elution Buffer or TE into the center of the column matrix and let stand at 60°C for 3 minutes.
> Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.

Troubleshooting

Problem	Comments and suggestions
Low yield of DNA	
>Incompletely lysed sample	1Decrease the sample amount prior to use.
>Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the Wb2 Buffer prior to use.
>Buffer EL pH is too low	Check the pH.
>Buffer EL not pre-heated to 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 DNA isolation. Perform the extraction using fresh materials whenever possible.
>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 WB2, 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 WB1 first, and then proceed to the wash step with Buffer WB2.