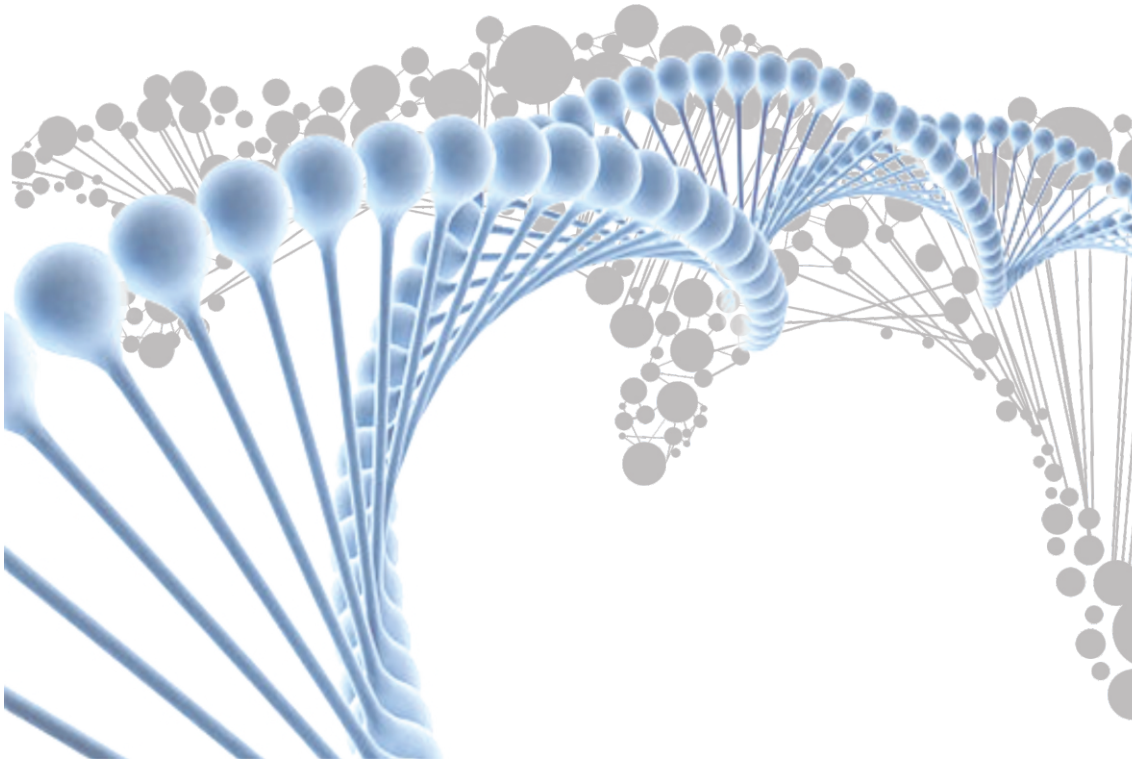
MGK-T0100 & MGK-T0300

Genomic DNA Isolation --Tissue



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Genomic DNA Isolation Kit (Tissue)

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: 30 mg of animal tissue or 25 mg of FFPE tissue

Introduction:

The EBL Genomic DNA Isolation Kit (Tissue) was designed specifically for genomic DNA isolation from fresh, frozen or FFPE tissue samples. Its unique buffer system ensures total DNA with high yield and good quality from samples while its spin column system purifies or concentrates DNA products previously isolated with buffers. The entire procedure can be completed within 60 min without phenol / chloroform extraction. Purified DNA is then suitable for use in PCR or other enzymatic reactions.

About the kits:

Cat. No.	MGK-T0100	MGK-T0300
SD Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs
TL Buffer	35 ml	95 ml
PR Buffer	12 ml	35 ml
W1 Buffer	45 ml	125 ml
W2Buffer	15 ml	25 ml x2
Elution Buffer	10 ml	30 ml

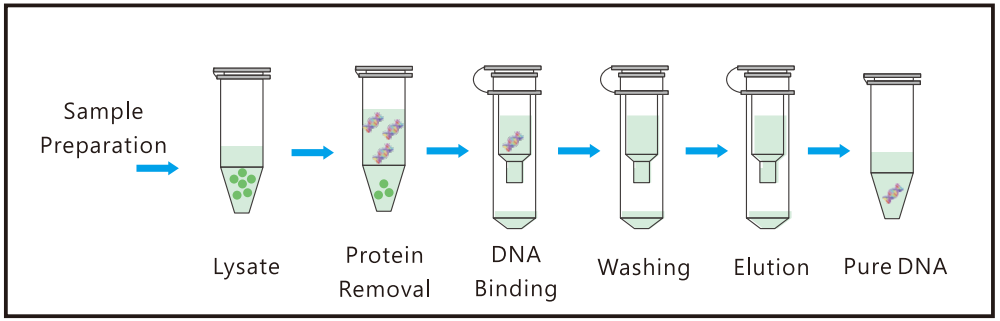
NOTE:

1. Add ethanol (96–100%) to Buffer W2 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

Procedure:



Protocol:

Step 1. Sample Preparation

Fresh Tissue

> Cut off 30 mg of fresh animal tissue and grind the sample under liquid nitrogen to a fine powder using a mortar and pestle and transfer it to a 1.5 ml microcentrifuge tube.

Paraffin-embedded tissue

> Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
> Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
> Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
> Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
> Proceed with the Lysis Step.

Step 2. Lysis

> Add 300 µl of TL Buffer and 20 µl of Proteinase K (10mg/ml) to the tube from Step 1.
> Incubate at 60°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 60°C until it is clear.

#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 PR Buffer to the sample from Step 2 and shake vigorously.

> Centrifuge at 14,000 x g for 3 minute. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

> Add 300 µl of absolute ethanol to the sample lysate and shake vigorously (break up any precipitate by pipetting but be careful to not let any pellet remain inside the pipette tip after you are done).

Step 4. DNA Binding

> Place a SD Column in a 2 ml Collection Tube.

> Transfer the mixture 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 W1 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 W2 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 W2 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	1. Use the required range or amount of starting materials to prepare the lysates. 2. Increase the digestion time. 3. Make sure that the tissue is completely immersed in the Buffer TL.
>Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the W2 Buffer prior to use.
>Incorrect elution conditions	Perform incubation at 75°C for 3 minutes with Elution Buffer before centrifugation. To recover more DNA, repeat the elution step.
>Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample at -80°C or in liquid nitrogen. The yield and quality of DNA isolated depends on the type and age of the starting material.
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	1. Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents). 2. Use fresh TAE or TBE electrophoresis buffer.
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.