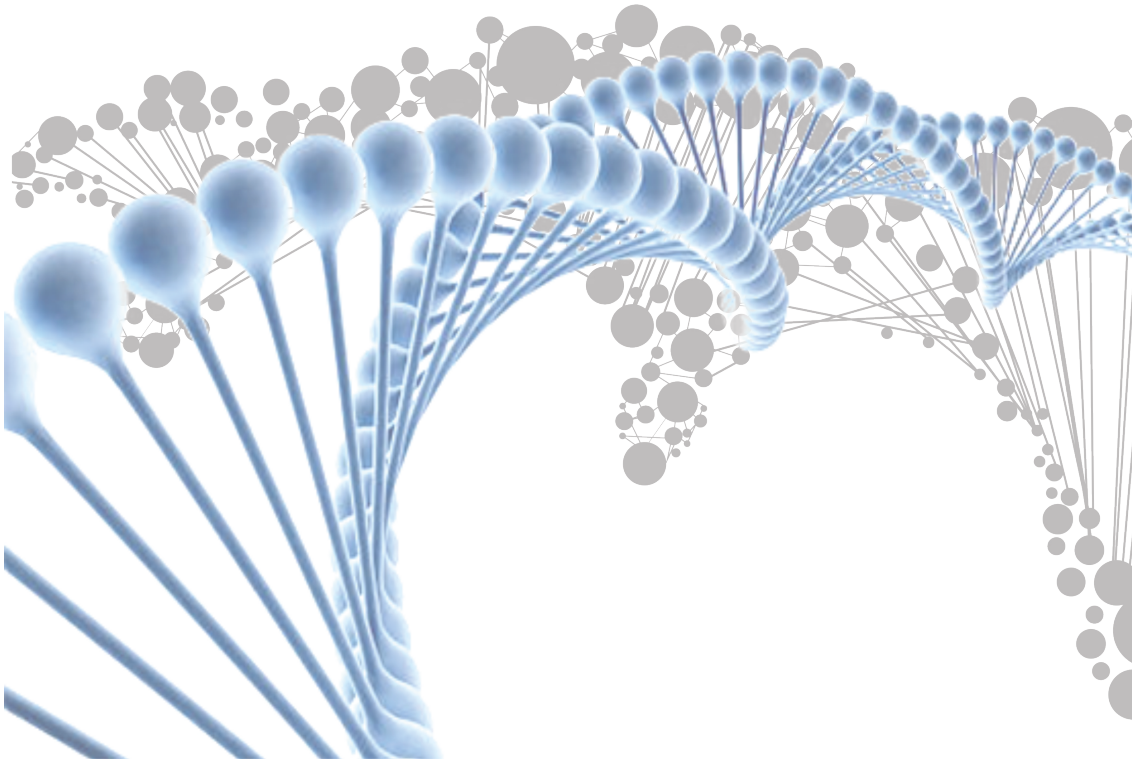
MGK-P0100 & MGK-P0300

Genomic DNA Isolation --Plant



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Genomic DNA Isolation Kit (Plant)

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: up to 100 mg of fresh plant tissue or 50 mg of dry plant tissue

Introduction:

The EBL Genomic DNA Isolation Kit (Plant) was designed specifically for genomic DNA isolation from Plant samples. This unique buffer system ensures total DNA with high yield and good quality from samples and the spin column system was designed to purify or concentrate DNA products which have been previously isolated using buffers. The entire procedure can be completed in 1 hour without phenol / chloroform extraction. Purified DNA is suitable for use in PCR or other enzymatic reactions.

About the kits:

Cat. No.	MGK-P0100	MGK-P0300
SD Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs
PL Buffer	55 ml	155 ml
W1 Buffer	45 ml	125 ml
W2Buffer	15 ml	25 ml x2
Elution Buffer	10 ml	30 ml

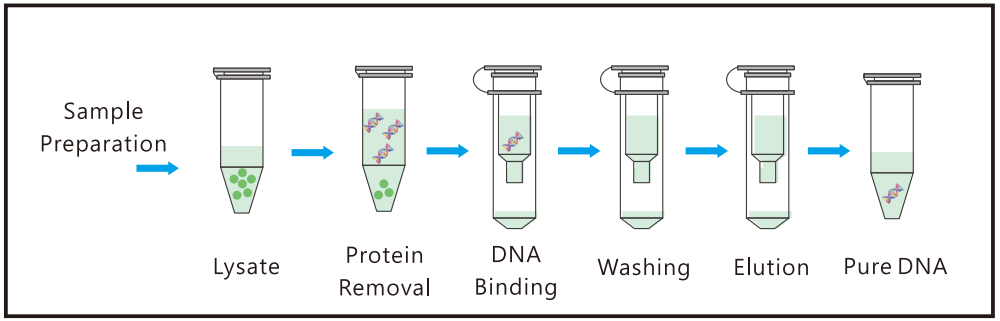
NOTE:

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

> Cut off 50-100 mg of fresh plant tissue or 25 mg of dry plant tissue. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 2. Lysis

> Add 500 µl of PL Buffer and 0.5 µl of RNase A (50 mg/ml) to the sample in the mortar and grind the sample until it is completely dissolved.

> Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. Incubate at 75℃ for 30 minutes (invert the tube every 10 minutes).

> Centrifuge at 14-16,000 x g for 5 minutes. Transfer the supernatant to a new 1.5 ml micro-centrifuge tube.

#Pre-heat the Elution Buffer to 75℃ for Step 6 DNA Elution.

Step 3. DNA Binding

> Add the same volume of Isopropanol to the clear supernatant from the previous step and vortex immediately for 5 seconds. (eg. add 350 µl Isopropanol to 350 µl supernatant)

> 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 4. 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 PL.
>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.