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Gene-Spin™ Genomic DNA Isolation Kit

Product Description: **Cat# GD112-V2**

The **Gene-Spin™ Genomic DNA Isolation Kit** is designed for rapid purification of genomic DNA from various animal and plant tissues, culture cells and blood sample. The method is based on a spin column format, after cell lysis and subsequent proteinase K digestion, the nucleic acids are absorbed into the specially silica membrane, the genomic DNA is then eluted by preheated water or TE buffer. DNA purified with this kit is suitable for various applications, including PCR and restriction enzyme digestion.

Components: ***Store at Room Temperature**

The kit contains reagents sufficient for 50 preparations.

- Extraction Solution - 20 mL (10 mM Tris, 100mM EDTA, *p*H8.0, 0.5% Triton X-100)
- DNA binding buffer - 20 mL (8M Guanidine HCl)
- Wash Solution - 16 mL wash solution. Please add 64 mL up to 95% ethanol before using.
- Proteinase K powder - 1btl (5 mg)
- Gene-Spin spin columns - 50 pcs
- Collection tubes - 50 pcs

Before starting:

- **Add 220 µL sterile H_2O into proteinase K bottle, shake well to completely dissolved. Store at 4^0C or -20^0C.**
- **For isolation DNA from blood sample, prepare RBC lysis buffer as follow: 150 mM NH_4Cl, 0.1 mM EDTA and 10 mM $KHCO_3$ Store at 4°C**

General Protocol:

Materials to be supplied by the user

- **For tissue grinding: Small homogenizer (Fisher Tissue Tearor, Polytron or Turrax.) Alternatively, mortar and pestle.**
- **Trypsin (for adherent tissue culture cells only)**
- **PBS buffer**
- **Water bath, 56°C and 70°C.**

a. Tissue culture cells

1) Harvest the cells (for adherent cells, trypsinize the cells before harvesting) and transfer to 1.5 mL centrifuge tube.
2) Centrifuge at 14,000 g for 10s, remove the supernatant.
3) Wash by 200 µL PBS, centrifuge again at 14,000 g for 10s, remove the supernatant.
4) Add 50 µL PBS and resuspend the pellet by pipetting or vortex.
5) Add 350 µL **Extraction buffer** and proceed to Step 2.

b. Animal tissue

1) Using homogenizer: add ice cold 350 µL extraction buffer to 10-50 mg tissue and homogenize for 10 seconds, then transfer to 1.5 mL centrifuge tube, proceed to Step 2.
2) Using mortar and pestle: pre-chill the sample and mortar and pestle at -70°C at least 1 hour then grind the tissue to powder or just grind the tissue with liquid nitrogen in the mortar. Transfer the ground tissue to 1.5 mL centrifuge tube, then add 350 µL **Extraction buffer** and proceed to step 2.

c. Blood sample

1) Add 4X volume of ice cold RBC lysis buffer to whole blood sample (<300 µL), incubate at RT for at least 5 min or until red blood cells complete lysis.

2) Discard the supernatant as complete as possible, wash the nuclei by 200 µL PBS.
3) Add 50 µL PBS and resuspend the pellet by pippetting or vortex.
4) Add 350 µL **Extraction buffer** and proceed to Step 2.

d. Plant tissue

1) Prechill the sample (<50 mg) and mortar and pestle at –70 °C at least 1 hour then grind the tissue to powder or just grind the tissue with liquid nitrogen in the mortar. Transfer the ground tissue to 1.5 mL centrifuge tube, then add 350 µL **Extraction buffer** and proceed to step 2.

e. bacteria cells

1) Re-suspend the pellet cells with 20 mM Tris-HCl , 2 mM EDTA, 1% Triton X-100, 20 mg/mL Lysozyme or 0.2 mg/mL lysostaphin , *p*H 8.0 (10^9 cells add 100 µL)
2) Incubation for 30 – 60 min at 37°C
3) Following the procedure step 1 (v) add 350 µL **Extraction Buffer**

1. Add 4 µL **proteinase K stock solution**, mix by gentle vortexing.
2. Incubate at 56°C in water-bath or incubator until complete lysis (~1-3 h).

   ** Using shakable water-bath or incubator may shorten the incubation time.*

   ** The solution will become opaque at 56°C, this is because of Triton X-100 in the extraction buffer, which will not affect the reaction and will return clear when cool to room temperature.*
3. Add 300 µL **DNA binding buffer** to solution, mix well.

   ** Some tissue debris (i.e. zebrafish bone or plant fiber..) may not be digestible, it is important to remove the debris by centrifuging at 14,000g for 5 min before loading on column, since these debris will clot the column.*
4. Apply the solution to spin column with collection tube, spin at 14,000 rpm for 1 min,

discard the flow-through.

5. Wash twice with 700 µL wash solution, discard the flow-through.
6. Centrifuge for 5 min at top speed to remove any residual trace of ethanol.

   ** Some trace of ethanol may still remain, it is preferable to incubate the spin column at 60 °C oven for 5-10 mins to evaporate all the ethanol before eluting the DNA.*
7. Remove the **collection tube** and place the column in a new microcentrifuge tube. Add preheated 70°C of 50-100 µL H_2O or TE.
8. Elute the DNA by centrifugation for 1 min, elute again to have more DNA. Store DNA at -20°C.

   ** Incubate the column in 60-70°C oven before centrifuging will lead to a better yield.*

For Research Using Only.
Please do not hesitate to contact us if you have any questions.

Manufactured for and distributed by Protech Technology Enterprise Co., Ltd.
TEL : +886-2-2655-7677 / FAX : +886-2-2655-7601 / Toll Free : 0800-231-530
E-MAIL: service@bio-protech.com.tw ; tech@bio-protech.com.tw
WEB: http://www.bio-protech.com.tw