شرکت تحقیقاتی تولیدی پویا ژن آزما

POUYA GENE AZMA Co. (PGA)

PGA Blood, Tissue and Cell Culture DNA purification kit

Catalog No. PD125-050 **Quantity: 50 Prep**

Storage :RNase A and proteinase K at -20oC for one year; others at room temperature (15-25oC) for one year.

Kit Description:

The PGA Blood ,Tissue and Cell Culture DNA purification Kit offers a column base method for purification of high molecular weight genomic, mitochondrial or viral DNA from Blood, cultured cells or animal tissues. The high quality extracted DNA is ready for use in PCR, restriction digests, and other downstream applications. This Kit is safe and user-friendly because it avoids the use of organic solvents like phenol and chloroform.

Advantages

Use of the PGA Blood, Tissue and Cell Culture DNA purification Kit to isolate genomic DNA provides the following advantages:

- Rapid and efficient purification of genomic DNA from mini quantities of tissue in less than 15 minutes following sample preparation and lysis
- Simple lysis of tissues with Proteinase K without the need for any mechanical lysis
- Minimal contamination with RNA
- The purified genomic DNA demonstrates improved downstream performance in applications including PCR, restriction enzyme digestion, and Southern blotting

KIT CONTENTS:

Mini spin columns	50x
Collection tube	50 X
Buffer R - Cell Resuspension Buffer	10 ml
Buffer L - Cell Lysis Buffer	12.5 ml
Buffer EW – Extra wash Buffer	17.5
Buffer W - Column Wash Buffer	25 ml
Buffer EB - Elution Buffer	5 ml
RNase A (20 mg/ml)	100 µl
Proteinase K (20 mg/ml)	250 µl
Protocol Handbook	1

Safety Information

Follow the safety guidelines below when using the Tissue Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

LABORATORY PROTOCOL:

Before Starting

Perform the following before beginning:

- Set a water bath at 55° C.
- For each sample, mix 200 µl of R buffer and 5 µl of Proteinase K. When isolating DNA from multiple samples, scale up the volume of reagents used and prepare a master.

1. Preparing Cells

-Tissue Dissociation: Transfer 10-20 mg of tissue to a 1.5 ml microcentrifuge tube and use a micropestle to grind the tissue a few times.
- Cell Dissociation: Transfer 200-500 µl suspention pop smear or 3-5 x 10^6 of cell culture to 1.5 ml microcentrifuge tube and centrifuge 5 minutes at maximum speed. Remove and dispose of liquid by pipette.
- Blood : Whole blood must be collected in EDTA (1mg/ml)- to prevent clotting and DNA degradation. Add 100 µl whole blood to 1.5 ml microtube, and then follow the laboratory protocol.
-sera: Add 100 ul of serum or plasma in 1.5 ml micro centrifuge tube, and then follow the laboratory protocol. Usage of fresh sera samples is recommended.
2:Add 200 µl mixed R buffer (see above) and resuspending of precipitate in it. Mix by vortex or by pipette and incubate at 55°C for 30-50 minutes (mix occasionally to aid in digesting). Some tissues require less or more ProK and incubation time to aid in digesting but blood need 5 minutes incubation only.
3:Add 400 µl L buffer to tube and inverting 2 times gently and keep in room temperature for 5 - 10 minutes until produce clear Solution.
4:Add 2 µl of RNase A to the lysate. Pipet up and down gently 5 times or until a homogeneous solution is obtained. Incubate at room temperature for 5 minutes.
5. Transfer the solution to a spin column with collection tube.

6. Centrifuge the tube at 13.000 rpm for 1 min. Discard the flow through.

7. Add 650 µl of EW buffer (check to ensure you have added ethanol) to the column , centrifuge at 13000 rpm for 1 minut. Discard the flow through.
9. Wash the spin column with 400 µl of W buffer by centrifugation at 13.000 rpm for 1 min. Discard flow-through.

10- Place the spin column in a clean microcentifuge tube, add 30-100 µl of E buffer or distilled water directly to the center of the column matrix. Incubate the column at room temprature for 1 minute. Centrifuge the column at 12000 rpm for 1 minute to elute DNA. The isolated plasmid DNA is ready to use or can be stored at -20°C.