

Description

Genomic DNA system uses the silica-gel-membrane technology for simple and fast isolation of Genomic DNA without phenol/chloroform. Homogenization is not necessary since tissues are directly lysed by Proteinase K. The buffer system is optimized to allow selective binding of DNA to the silica-gel membrane. The simple centrifugation protocol completely removes contaminants such as proteins, divalent cations, and secondary metabolites. Pure DNA is then eluted in water or low-salt buffer, ready to use. The kit is suitable for anticoagulated blood. 400 µl whole blood yield 3-10 µg high pure genomic DNA. The purified DNA can be used in common downstream applications such as sequencing, restriction digestion, etc.

Applications

Purified DNA is free from contaminants and enzyme inhibitors, and typically has A260/A280 ratios between 1.7 and 1.9, and is suitable for applications such as:

- Restriction digestion
- PCR
- Labeling
- Southern hybridisation
- Library construction

Feature.

- High Efficient – 3-10 µg genomic DNA from 400 µl whole blood
- Safe - no phenol/chloroform extraction step.
- High purity - Purified DNA is ready for downstream application such as PCR, restriction digestion.

Store

store Proteinase K at -20, other reagents can be store at room temperature for up to 1 year. Any precipitate in the Solution DDS and solution DMS can be re-dissolved by incubating at 37°C before use.

Note

- Prior to the initial use of the kit, dilute the Wash Buffer DPE with ethanol (96-100%):

	DN1131(50preps)	DN1132(100 Praps)
Wash Buffer VPE	15 ml	15 ml x2
Ethanol	45 ml	45 ml x2
Total Volume	60 ml	60 ml x2



Blood Genomic DNA Extraction Kit

For research use only

Kit Content

Content	DN1131	DN1132
Solution DRS	80 ml	160 ml
Solution DV	15 ml	30 ml
Solution DMS	20 ml	40 ml
Proteinase K	1 ml	2 ml
Wash Buffer DPS	30 ml	60 ml
Wash Buffer DPE	15 ml	30 ml
Elution Buffer DTE(10 mM Tris-HCl, pH 8.5)	5 ml	10 ml
Spin Column	50 each	100 each

7. Repeat step 6 again.

8. Centrifuge for 3 min at 12,000 rpm to dry the column membrane. Discard flow-through and collection tube.

Note It is important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution. Following the centrifugation step, remove the spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 12,000 rpm.

9. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer TE (prewarm to 60) directly onto the membrane. Incubate at room temperature for 2 min.

Note • Elution buffer DTE can be replaced by deionized water. But the PH should be 8.0-8.5.

• Prewarm Elution Buffer DTE to 60 can increase the yield of genomic DNA.

10. Centrifuge for 2 min at 12,000 rpm to elute. The tube contains the purified DNA.

Store the DNA at -20 °C.

After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

- Examine the solution for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
- Wear gloves when handling the Solution DMS as this solution contains irritants.
- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out in a table-top microcentrifuge at >12000 g (10,000-14,000 rpm, depending on the rotor type)

Protocol

1. Add 400µl anticoagulated blood into a 1.5 ml microcentrifuge tube, add 2 volume of Solution RS to the tube. Mix thoroughly by vortexing. Centrifuge for 3 min at 5,000 rpm, discard the supernatant. The pellet is white or pink.

Note • If the pellet's colour is dark red, it suggests that the lyse process is not complete, you may add another 500 µl Solution RS to lyse the sample.

• Blood from mammals contains nonnucleated erythrocytes. Blood from animals such as birds, fish, or frogs contains nucleated erythrocytes. For blood with nonnucleated erythrocytes, 400µl blood can yield 3-10µg genomic DNA. For blood with nucleated erythrocytes, the volume of blood do not exceed 20 µl per tube. 20 µl whole blood can yield 40 µg genomic DNA.

2. Add 200 µl Solution DS. It is essential that the sample and Solution DV are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

Optional If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 5 min at room temperature. RNase A (100 mg/ml) can be purchased separately.

3. Add 20 µl Proteinase K and 220 µl Solution DMS, Mix thoroughly by vortexing. Incubate at 65°C for 10 min to yield a homogeneous solution.

4. Add 220 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution. Pipet the mixture from step 3 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 12,000rpm for 1 min. Discard flow-through.

Note Genomic DNA is adsorbed on the silica membrane of the column in this step.

5. Add 500 µl Wash Buffer DPS, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.

6. Add 500 µl Wash Buffer DPE, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.

Note Wash Buffer DPE must previously diluted with ethanol (96-100%).

شرکت ویژن پارس دلتا

شماره های تماس: 02155433092

02155423112

Email: v.gene.pars@gmail.com

Website: www.vgenepars.ir