Description

Marine Animal Tissue Genomic DNA Purification Kit provides a simple and rapid method for high quality genomic DNA purification from tissues(either fresh or frozen at -70°C until use) of marine animal, such as fish, shrimp, shellfish or crab. The 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 typical yield of genomic DNA is 3-35 µg from 10 mg of tissue. The purified high molecular weight genomic DNA is suitable for direct use in all common molecular biology applications: PCR, restriction digestion, cloning, DNA sequencing and Southern blot analysis.

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**
- **Library construction**

Feature.

- **Fast - procedure takes only 30 min.**
- **High Efficient – 3-35 µg of genomic DNA from 10 mg of tissue or 1 x 106-107 cultrue cells.**
- **Safe - no phenol/cholroform 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%):**

	DN1171(50preps)
Wash Buffer DPE	15 ml
Ethanol	45 ml
Total Volume	60 ml

Marine Animal Tissue Genomic DNA Extraction Kit

Cat No: DN1171 Volume: 50 preps

For research use only

Kit content

Content	DN1171
Solution DDS	15 ml
Solution DMS	20 ml
Proteinase K	1 ml
Wash Buffer DPS	30 ml
Wash Buffer DPE	15 ml
Elution Buffer DTE (10 mM Tris-HCl,pH 8.5)	5 ml
Spin Column	50 each

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 MS 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. Marine animal tissue (either fresh or frozen at -70°C until use) can be processed by freezing with liquid nitrogen and grinding into a fine powder using a mortar and pestle. Add 10mg of this tissue powder to a 1.5ml microcentrifuge tube. Do not exceed 10 mg or it will reduce the lyse efficiency.

2. Add 200 µl Solution DDS. Vortex vigorously to resuspend cells.It is essential that the sample and Solution DDS 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. Mix thoroughly by vortexing. Incubate at 55 and inverting at times till a clear homogeneous solution is produced, spin down the water beads from the wall of the tube.

4. Add 220 µl Solution DMS, Mix thoroughly by vortexing. Incubate at 65°C for 10 min to yield a homogeneous solution. Spin down the water beads from the wall of the tube.

5. Add 220 µl ethanol (96–100%) to the sample, and mix thoroughly by inverting.

A precipitate may appear. Pipet the mixture from step 4 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 12,000rpm for 1 min. Discard flow-through.

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

7. 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%).

8. Repeat step 7 again.

9. 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. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer DTE (prewarm to 60)directly onto the membrane. Incubate at room temperature for 2 min, and then centrifuge for 2 min at 12,000 rpm to elute. The tube contains the purified DNA. Store the DNA at -20 。

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.

شرکت ویژن پارس دلتا
شماره های تماس: 02155433092
02155423112
Email: v.gene.pars@gmail.com
Website: www.vgenepars.ir