

Cell/Tissue DNA Extraction Kit

(Solution)

A fast kit for the isolation of genomic DNA from cell/tissue

For laboratory research use only

DP1201 **50 preps**

Kit Content, Storage, and Stability

Content	Storage	50 preps
Nuclear Lysis Buffer	RT	33 ml
Protein Precipitation Buffer	RT	11 ml
DNA Dissolving Buffer	RT	10 ml
RNase A (10 mg/ml)	-20°C	100 ul

All reagents are stable for 12 months when stored properly.

Notes

1. Nuclear lysis buffer may form precipitation due to low storage temperatures. If necessary, dissolve the precipitation by 37°C water-bath and then cool to room temperature before use.
2. Protein precipitation solution may form precipitation. If necessary, dissolve the precipitation by 37°C water-bath, or harvest upper-liquid for use.
3. Please ensure the bottles of buffer are tightly capped when not in use, preventing reagents evaporating, oxidation and pH changing.

Principle

This kit is applied to fast extract DNA from cells or tissues. Nuclear lysis buffer splits cells with detergents or protease K and the genomic DNA is released. RNA is removed by RNase. Then protein precipitation solution precipitates and removes proteins selectively. Finally, the purified DNA is precipitated by isopropanol and then DNA is resuspended in DNA dissolving solution.

Features

1. Poisonous phenol etc not used.
2. Procedure is simple and fast, single sample can be completed in 60 min.
3. Stable results, high yield, high purity. The typical ratio of OD260/OD280 is 1.7~1.9, and the average length is up to 30kb-50kb, which can be applied for PCR, Southern-blot and digestions directly.

Notes

1. All the centrifugation steps can be performed at room temperature.
2. Please prepare 70% ethanol, isopropanol, PBS (used for cell), mortar or homogenizer (used for tissue), 0.5M EDTA and protease K (used for mouse tail snips) and water bath.
3. This kit is solution type, which can be proportionally amplified or narrowed to samples size.

Procedure

1. Cultured tissue cells

a. cell collection

For cells grown in suspension, pellet 10^5 - 10^6 cells by spinning in a 1.5ml centrifuge tube.

For cells grown in a monolayer, harvest the cells by trypsin treatment.

- b. Centrifuge at 13,000 rpm for 10 sec. Discard the supernatant. Leave the pellet and 10-50 μ l culture at the bottom.
- c. Add 200 μ l Buffer PBS to resuspend cells. Repeat step b. Dispense the cells by vortex.
- d. Add 600 μ l nuclear lysis buffer. Beat up the cells thoroughly.
- e. Proceed the step 4.

2. Tissue (such as rat liver and plant leaves)

- a. Mince fresh or thawed tissue into small pieces or grind it into powders with liquid nitrogen. Transfer 10-20 mg tissue (40 mg plant leaves) to a 1.5ml centrifuge tube and add 600 μ l nuclear lysis buffer, mix by pepeating.
- b. Incubate at 65°C for 15-30 min or until tissue completely lysed. Mix several times during incubation.
- c. Proceed the step 4.

3. Tissues (Mouse tail snips)

- a. Add 120 μ l 0.5M EDTA (pH8.0) and 500 μ l nuclear lysis buffer into a 1.5 ml centrifuge tube. Mix thoroughly and keep on ice.
- b. Grind rat tail into powders with liquid nitrogen or take cells from peaked tail 0.5-1.0 cm in length. Transfer to a 1.5 ml centrifuge tube and add 600 μ l EDTA/Nuclear lysis buffer prepared in step a.
- c. Add 17.5 μ l Proteinase K (20 mg/ml), mix thoroughly.
- d. Incubate at 55°C for overnight or until tissue completely lysis. Mix gently to in incubation.

4. Add 1.8 μ l RNase A (10mg/ml) into cracking substance at a final concentration of 30 μ g/ml). Mix thoroughly and incubate for 15 min in 37°C to remove residues of RNA. Then cool to room temperature.

5. Add 300 μ l protein precipitation and vortex for 20-25 sec, some small protein masses appear after mixing. Put on ice for 5 min.

6. Centrifuge at 13,000 rpm for 5 min. White protein precipitation at the bottom of tube or in the surface of liquid will appear.

7. Carefully transfer supernatant to a new 1.5 ml centrifuge tube.

8. Add the equal volume of isopropanol (about 600 μ l) and overturn gently for 30 times till some white filamentous DNA precipitation appear.

9. Vertically place centrifuge tube, remove supernatant as much as possible, leaving the bottom white DNA precipitation. For better sedimentation of DNA is possible to centrifuge microtube at 12,000 rpm for 1 min

10. Add 1 ml 70% ethanol and mix. Centrifuge at 13,000 rpm for 1 min. Discard the supernatant.

11. Add 0.5ml 70% ethanol. Rinse DNA precipitation, centrifuge at 12,000 rpm for 1 min, discard supernatant thoroughly by absorbent paper and air dry for several minutes.

The DNA will be hard to dissolve because of excessive air dry. Don't leave too much ethanol in the microtube with DNA, as it could inhibit the downstream endonuclease reaction.

12. Add 100 μ l DNA (or adjust by concentration) dissolving buffer to dissolve DNA precipitation. Mix by tap the tube wall and incubate for 30 min at 65°C (no more than 1 h), or place stay over at room temperature or 4°C.

13. DNA could be stored at 2-8°C, or at -20°C for long term storage.

Troubleshooting

Problem	Possible causes	Advices
Low DNA yield	Lysis buffer improperly used	The sample to treat is not excessive
	Some tissue e.g. muscle and mouse tail snips are difficult to treat	The sample has to be grinded or homogenated fully
	DNA precipitation lost at washed	Discard the supermatant after the ethanol washing carefully.

A260/A280>1.9	RNA contamination	Increase the amount of RNase A or prolong the digestion time to 1h.
	DNA fragmentation,	Perform gently
No protein precipitation appear	The mixture don't cool to room temperature before add protein precipitation	Cool to room temperature or incubate for 5 min on ice ,then add protein precipitation
	No thoroughly mixed between protein precipitation solution and cracking mixture.	Vortex agitation for 25 s, vortex agitation will not cut off DNA Ice bath 5 min before centrifugation.
DNA length is less than 20kb	The sample is not fresh or improper storage	Use fresh samples
	Incorrect operation lead to genomic DNA shear protein contamination	It should not too violent when mixed, transfer it by large diameter pipette tips or mix DNA
A260/A280 <1.6	Dilute DNA with water when measure the value of A260/A280	Use TE buffer instead of water ,keep PH value >8.0
DNA precipitation hard to redissolution	Excessive air dry of DNA precipitation	No excessive air dry
Downstream digestion inhibited	DNA does not dry completely, too much ethanol left.	Open wide centrifuge tube, incubate several minutes in 65°C to volatilize ethanol