Cell/Tissue DNA Extraction Kit

(Spin-column)

For laboratory research use only

DP1901 50 preps

Kit Content, Storage, and Stability

Content	Storage	50 preps
Buffer TB	RT	22 ml
Buffer TL	RT	11 ml
Buffer CB	RT	11 ml
Buffer IR	RT	27 ml
Buffer WB	RT	15 ml
	Add ration ethanol before use	
Buffer EB	RT	15 ml
Isopropanol	RT	7 ml
Proteinase K (20 mg/ml) (for type II)	-20°C	20 mg (powder)
Spin-column AC	RT	50
Collection Tube (2 ml)	RT	50

All reagents are stable for 12 months when stored properly.

Notes

Please add 60 ml ethanol to 15 ml Buffer WB before first time, vortex adequately and then check it to avoid multi-adding!

Buffer CB or IR may precipitate under low temperature, incubate them at 37°C water-bath for a moment **until clear**, then cool down to RT for use.

Proteinase K is provided in freeze-dried powder for activity and transportation. Centrifuge a few seconds and **add 1ml sterile water to the tube.** Because frozen and melt repeatedly may affect enzyme activity, please aliquot and store under -20°C.

Please cap all reagents bottles tightly after use to prevent reagents from evaporating, oxidation and pH changing.

Principle

The Kit applies the unique binding buffer/ Proteinase K to rapidly lyse cells and inactivate cellualar nucleases, then DNA is selectively absorbed on silica membrane in high salt solution, after that serial of elution- centrifugation steps remove cellular metabolite and proteins. Finally low salt elution elutes purified genomic DNA from silica membrane

Features

1. Poisonous phenol etc not used.
2. Procedure is simple and fast, single sample can be completed in 30 min.
3. Multi-elution can ensure high-purified DNA, the typical ratio of OD260/OD280 is 1.7≈1.9, and the average length 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 RT*** and 13,000 rpm in traditional centrifuge.
2. Before use, please set water-bath at 70°C
3. Buffer CB and IR contains the stimulating compound, please wear latex gloves, and avoid skin, eyes and cloth to be contaminated. If that, please use water or physiological saline washing.

Procedure

REMEMBER - Please dilute 15 ml Buffer WB with 60 ml absolute ethanol.

1. Cultured tissue cells

a. Collect 10^5-10^6 suspended cells to a 1.5ml clean tube. For adherent cells, treat with Trypsin at first.
b. Spin at 13,000 rpm, 10 min to collect cells, discard the supernatant, leave over the cell crops and about 10-20 µl left over liquid.
c. Add 200 µl Buffer TB to resuspend and wash cells. Repeat step 1b, discard supernatant, suspend cells in 200µl Buffer TB again.
d. Add 200 µl buffer CB, then **overturn to mix thoroughly**, add 20 µl Proteinase K (20mg/ml), mix thoroughly, incubate at 70°C for 10 min.
e. Cool down to RT, add 100µl isopropyl alcohol, then **overturn to mix thoroughly**, maybe appear the flocculated precipitate.
f. Put last step solution and the flocculated precipitate into a Spin-column AC (place the spin-column to collection tube), then 10.000 rpm 30 sec and discard flow-through**.**
g. Continue to do follow step 4.

2. Propagation tissue (such as rat liver and brain)

a. Cut the fresh or thawy tissue into small blocks with scalpel (for increasing yield) or make it into powder in liquid nitrogen, transfer 20-50 mg power into a 1.5ml tube containing 200 µl Buffer TL, mix by pepetting.
b. Add 20µl Proteinase K (20mg/ml), turnover and thoroughly mix.
c. Incubate at 55°C for 60 min and till thorough digestion, mix gently to help digest.
d. Add 200µl buffer CB, then **overturn to** mix thoroughly, incubate at 70°C for 10 min.
e. Cool down to RT, add 100 µl Isopropanol, then **overturn to mix thoroughly**.
f. Transfer supernatant into a spin-column AC (place the Spin-column to collection tube), then centrifuge at 10,000rpm for 30 min, discard flow-through**. Please do not take water-fast matter in the mixed solution into Spin-column AC, otherwise it will block Spin column AC!**
g. Continue to do follow step 4.

3. Animal tissue (rat tail)

a. Grand rat tail into powder in liquid nitrogen or take cells from peaked tail in 0-2 cm range and sheer the cells into small blocks with scalpel. Then transfer cells into a 1.5 ml tube containing 200 µl Buffer TL, mix by peptiting using big caliber tips.
b. Add 20µl Proteinase K (20 mg/ml), turnover and thoroughly mix.
c. Incubate at 55°C for 3 hours and till thoroughly digest, mix gently to help digest.
d. Beat upon the above solution several times with 1ml injector without needle.
e. Add 200 µl Buffer CB and 100 ul Isopropanol, then **overturn to mix thoroughly**.
f. Centrifuge at 13,000rpm for 5 min, transfer the supernatant into a spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000 rpm for 30 min, discard flow-through**.**
g. Continue to do follow step 4.

Take the above steps, please pay attention on solution mixing, otherwise will affect yield! If necessary, vortex for 15 sec.

4. Add 500 µl Buffer IR, centrifuge at 12,000 rpm for 30 sec, discard flow-through.

5. Add 700 µl Buffer WB (**please check if ethanol been added!**), centrifuge at 12,000 rpm for 30 sec, discard flow-through.

6. Add 500 µl Buffer WB, centrifuge at 12,000 rpm for 30 sec, discard flow-through.

7. Place the Spin-column AC back to collection tube, centrifuge at 13,000 rpm for 2 min.

8. Transfer the Spin-column AC to a clean tube, add 100 µl Buffer EB (incubated in 65-70°C water-bath), stand for 3-5 min at RT. Centrifuge at 12,000 rpm for 1 min. Take flow-through back the Spin-column AC, stand for 3-5 min at RT, centrifuge at 12,000 rpm for 1 min.

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The more elution volume, the more DNA yield. If you need high concentration, you can appropriately reduce elution volume. If the elution is less than 50ul, elution efficient will be decreased and DNA yield will be low.

9. Keep DNA at 2-8 ^{o}C. For long-term storage, please keep at -20°C.

Troubleshooting

Problem	Possible Reason	Advices
Low extraction DNA	Block is too big, caused not enough digestion by Proteinase K	To cut tissue more smaller; elongate digestion time even overnight; after former digestion then increase 20μl Proteinase K again and incubation at 55 ^{o}C for 1hour
	Proteinase K may be inactive	Please store in per dose under -20°C, avoiding frozen and melt repeatedly
	Lysis not adequately, or mixed not enough with isopropyl alcohol	Add Buffer CB and Proteinase K, then **overturn to mix thoroughly.** Add isopropyl alcohol, and then **overturn to mix thoroughly** then put in spin column. If too sticky, vortex for 15 seconds.
DNA in tissue degrading	Degraded by cellular nuclease in tissue	Please store sample at -20°C before treatment and do not use too much
No Product	Not add ethanol to Buffer WB	**Add ration ethanol before use.**
Low elution DNA percent	Ethanol in spin-column or collecting tube bottom	Ensure do step 7, or affect the elution efficient
	Use water or other solution replace buffer EB	Please read carefully step 8, just use Buffer EB
A260 too high	Silica membrane eluted, influence A260 value	Centrifuge at 13,000 rpm for 1 minute, carefully using the supernatant
DNA digestion inhibition	Silica membrane eluted, inhibit digestion	Centrifuge at 13,000 rpm for 1 minute, carefully using the supernatant
	Ethanol in spin-column or collecting tube bottom	Ensure do step 7, then air dry in RT for a moment