BIOCYTOGEN

Advancing Life Science Researches

Cell/Tissue DNA Extraction Kit
(Spin-column)

Cat. #: **DP1901 (50 preps)**
DP1902 (100 preps)
DP1903 (200 preps)

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I. Kit Content Storage and Stability

Content	Storage	**50** preps （**DP1901**）	**100** preps （**DP1902**）	**200** preps （**DP1903**）
Buffer TB	RT	22 ml	44 ml	88 ml
Buffer TL	RT	11 ml	22 ml	44 ml
Buffer CB	RT	11 ml	22 ml	44 ml
Buffer IR	RT	27 ml	50 ml	100 ml
Buffer WB	RT	15 ml	25ml	50ml
		Add ration ethanol before use.		
Buffer EB	RT	15 ml	20 ml	40 ml
Isopropanol	RT	7 ml	15 ml	30 ml
Proteinase K （20mg/ml） （for type II）	-20	20mg freeze-dried powder	20mg×2 freeze-dried powder	20mg ×4 freeze-dried powder
Spin-column AC	RT	50 pcs	100 pcs	200 pcs
Collection Tube （2ml）	RT	50 pcs	100 pcs	200 pcs

All reagents are stable for 12 months when stored properly.

Notes:

1. ***Please add 60ml ethanol to 15ml Buffer WB before first time, vortex adequately, and then check it to avoid multi-adding!***
2. Buffer CB or IR may precipitate under low temperature, incubate them at 37 water-bath for a moment ***until clear,*** then cool down to RT for use.
3. ***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.
4. Please cap all reagents bottles tightly after use to prevent reagents from evaporating, oxidation and pH changing

II. Principle

The Kit applies the unique binding buffer/ Proteinase K to rapidly lyse cells and inactivate cellualar nuclease, then DNA selectively absorbed on silica membrane in high salt solution, after that take a serial of elution- centrifugation step to remove cellular metabolite and proteins etc. Finally use low salt elution to elute purified genome DNA from silica membrane.

III. Features

1. The silica membrane in the spin-column comes from the world-famous company.
2. Poisonous phenol etc not used.
3. Procedure is simple and fast, single sample can be completed in 30 min.
4. 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.

IV.Notes

1. ***All the centrifugation steps can be performed at RT*** and 13,000 rpm in traditional one, for example Eppendorf 5415C and the similar.
2. Before use, please set water-bath at 70 .
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.

V. Procedure

Please add 60ml ethanol to 15ml Buffer WB before use!

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. 13,000rpm, 10 min to collect cells, discard the supernatant, leave over the cell crops and about 10-20µl leftover liquid.
   c. Add 200µl Buffer TB to suspend and wash cells, repeat step 1b, discard supernatant, suspend cells in 200µl Buffer TB again.
   d. Add 200µl buffer CB，then **overturn to mix thoroughly**，add 20µl Proteinase K (20mg/ml)，mix thoroughly，incubate at 70 water-bath 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 10000rpm 30 min, 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 have it into powders in liquid nitrogen, transfer 20-50mg power into a 1.5ml tube containing 200 µl Buffer TL, mix by peptiting.
   b. Add 20µl Proteinase K (20mg/ml), acutely turnover and thoroughly mix.
   c. Incubate at 55 for 60 min or till thorough digestion, mix gently to help digest.
   d. Add 200µl buffer CB，then **overturn to** mix thoroughly，incubate at 70 water-bath for10 min.
   e. Cool down to RT , add 100µl 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 will block Spin column AC!**
   g. Continue to do follow step 4.

3. Animal tissue (rat tail)

   a. Grand rat tail into powders 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 1.5ml tube contained 200µl Buffer TL, mix by peptiting using big caliber tips.
   b. Add 20µl Proteinase K (20mg/ml), acutely turnover and thoroughly mix.
   c. Incubate at 55 for 3 hours or till thoroughly digest, mix gently to help digest.
   d. Beat upon the above solution several times with 1ml injector without needle.
   e. Add 200µl Buffer CB and 100ul Isopropanol, then **overturn to mix thoroughly**.
   f. Centrifuge at 13,000rpm for 5min, transfer the supernatant into a

spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000rpm for 30min, discard flow-through.

g. Continue to do follow step 4.

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

4. Add 500μl Buffer IR，centrifuge at 12,000 rpm for 30sec，discard flow-through.
5. Add 700μl Buffer WB（**please check if ethanol been added!**），centrifuge at 12,000 rpm for 30sec，discard flow-through.
6. Add 500μl Buffer WB，centrifuge at 12,000 rpm for 30sec，discard flow-through.
7. Place the Spin-column AC back to collection tube，centrifuge at 13,000 rpm for 2min.
8. Transfer the Spin-column AC to a clean tube, add 100μl Buffer EB (having been incubated at 65-70 water-bath), stand for 3-5 min in RT. Centrifuge at 12,000 rpm for 1 min. Take flow-through back the Spin-column AC, stand for 3-5 min in RT, centrifuge at 12,000 rpm for 1 min.

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

9. Keep DNA at 2-8 . For long-term storage, please keep at -20 .

VI. Troubleshooting

Problem	Possible Reason	Advices
Low extraction DNA percent	Blocks 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 for 1hour .
	Proteinase K may be inactive	Please store in per dose under -20, avoiding frozen and melt repeatedly.
	Lysis not adequately, or mixed with isopropyl alcohol not enough	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 degraded	Degraded by cellular nuclease in tissue.	Please store sample at -20 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 efficiency.
	Use water or other solution replace buffer EB.	Please reading carefully step 8, just use Buffer EB.
A_{260} too high	Silica membrane eluted, influence A_{260} value.	Centrifuge at 13,000 rpm for 1 minutes, carefully using the supernatant.
DNA digestion inhibition	Silica membrane eluted, inhibit digestion	Centrifuge at 13,000 rpm for 1 minutes, carefully using the supernatant.
	Ethanol in spin-column or collecting tube bottom.	Ensure do step 7， then air dry in RT for a moment.