**Note:** *for laboratory research use only*

# Cell/Tissue DNA Extraction Kit
# (Spin-column)

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

Signalway Biotechnology

## Kit Content Storage and Stability

| **Content** | **Storage** | **50** preps<br>（**DP1901**） | **100** preps<br>（**DP1902**） |
|---|---|---|---|
| Buffer TB | RT | 22 ml | 44 ml |
| Buffer TL | RT | 11 ml | 22 ml |
| Buffer CB | RT | 11 ml | 22 ml |
| Buffer IR | RT | 27 ml | 50 ml |
| Buffer WB | RT | 15 ml | 25 ml |
| | | ***Add 60 ml ethanol before first use*** | ***Add 100 ml ethanol before first use*** |
| Buffer EB | RT | 15 ml | 20 ml |
| Isopropanol | RT | 7 ml | 15 ml |
| Proteinase K (20 mg/ml) (for type II) | -20°C | 20 mg<br>(freeze-dried powder) | 20 mg×2<br>(freeze-dried powder) |
| Spin-column AC | RT | 50 pcs | 100 pcs |
| Collection Tube (2ml) | RT | 50 pcs | 100 pcs |

***All reagents are stable for 12 months when stored properly.***

## Notes

1. ***Add ethanol to Buffer WB before use; mix adequately and then check the box on the label indicating ethanol was added!***
2. Buffer CB or IR may precipitate at low temperature. Incubate the buffer at 37°C **until the buffer is clear**, and then let the buffer cool to RT before 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.*** Frozen and melt repeatedly may affect enzyme activity, please aliquot and store under -20°C.
4. Keep the reagent lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

## Principle

The kit uses the unique binding buffer/Protease K to rapidly lyse cell and inactivate cellular nuclease. DNA is selectively adsorbed to silica membrane in high salt solution. Cellular metabolite and proteins are removed by serial of elution and centrifugation steps. Finally, low salt elution buffer elutes purified DNA from silica membrane.

## Features

1. Do not contain poisonous phenol. No need for ethanol precipitation of DNA.
2. Rapid and convenient.
3. Multi-elution steps ensure high-purified DNA. The typical ratio of OD260/OD280 is 1.7～1.9, and the average length is up to 30-50 kb, which can be applied for PCR，Southern-blot, and digestions directly.
4. Excellent yield.

## Notes

1. ***All the centrifugation steps can be performed at RT*** and 13,000 rpm in traditional micro-centrifuge machine, such as Eppendorf 5415C.
2. Please set water-bath at 70°C before use.
3. Buffers CB and IR contain a corrosive compound; please wear latex gloves to avoid direct contact with skin, eyes, and clothes. If contact occurs, wash with water or physiological saline.

## Procedure

***Add absolute ethanol to Buffer WB before use!***

1. Cultured tissue cells
   a. Collect $10^5$-$10^6$ suspended cells to a 1.5 ml clean tube. For adherent cells, treat with trypsin.
   b. Centrifuge at 13,000 rpm for 10 minutes to collect cells, and then discard the supernatant (with 10-20 µl leftover liquid).
   c. Add 200 µl Buffer TB to suspend and wash cells, repeat step 1-b, discard supernatant, and suspend cells in 200 µl Buffer TB again.
   d. Add 200 µl buffer CB，then invert the tube to mix thoroughly. Add 20 µl proteinase K (20 mg/ml)，mix thoroughly，incubate at 70°C water-bath for 10 minutes.
   e. Cool down to RT. Add 100 µl isopropanol，and then invert the tube to mix thoroughly. Flocculated precipitation may occur.
   f. Centrifuge at 13,000 rpm for 5 minutes, transfer the supernatant into a spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000 rpm for 30 seconds, discard flow-through**.**
   g. Continue the extraction process at 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 grind it into powders in liquid nitrogen. Transfer 20-50 mg tissue blocks or powders into a 1.5 ml tube containing 200 µl Buffer TL. Mix by peptiting.

   b. Add 20 µl proteinase K (20 mg/ml), acutely invert and thoroughly mix.

   c. Incubate at 55°C for 60 minutes or till thorough digestion. Mix gently to help digestion.

   d. Add 200 µl buffer CB，then overturn to mix thoroughly，incubate at 70°C water-bath for 10 minutes.

   e. Cool down to RT , add 100 µl isopropanol，then invert to mix thoroughly.

   f. Centrifuge at 13,000 rpm for 5 minutes, transfer the supernatant into a spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000 rpm for 30 seconds, discard flow-through**.**

   g. Continue extraction process at step 4.

3. Animal tissue (rat tail )

   a. Grind rat tail into powders in liquid nitrogen or take samples from peaked tail in 0-1.5 cm range and sheer the samples into small blocks with scalpel. Transfer the powders or the blocks into a 1.5 ml tube contained 200 µl Buffer TL, mix by peptiting using big caliber tips.

   b. Add 20 µl proteinase K (20 mg/ml), acutely invert for thorough mixing. ***If necessary, vortex tube for 15 seconds.***

   c. Incubate at 55°C for 3 hours or till thoroughly digest, mix gently to help digest.

   d. Beat upon the above solution several times with 1 ml syringe without needle.

   e. Add 200 µl Buffer CB and 100 µl isopropanol, and then invert tube to mix thoroughly. If too sticky, vortex for 15 seconds.

   f. Centrifuge at 13,000 rpm for 5 minutes, transfer the supernatant into a spin-column AC (place the spin-column to collection tube), then centrifuge at 10,000 rpm for 30 seconds, discard flow-through**.**

   g. Continue extraction process at step 4.

***Caution: Solution mixing during above steps is critical for high yield of DNA! If necessary, vortex the tube for 15 seconds.***

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

   ***The volume of elution buffer could be adjusted according to needs. Appropriate reduction of elution volume can increase concentration. However, the minimum volume is 50 µl. If the elution volume is less than 50 µl, elution efficiency and DNA yield can be affected.***
9. Keep DNA at -20°C or apply to down-stream reactions.

## Troubleshooting

| Problem | Possible Reason | Advices |
|---|---|---|
| Low extraction DNA percent | Block is too big, and proteinase K cannot digest it completely. | Cut tissue smaller; elongate digestion time even overnight; after first-time digestion, add 20 μl proteinase K again and incubate at 55°C for 1 hour |
| | Proteinase K may be inactive | Store in aliquots under -20°C. Avoid repeated freezing and thawing. |
| | Lysis not adequately, or mixed with isopropyl alcohol not enough | Add Buffer CB and proteinase K. Invert to mix thoroughly.<br>Add isopropanol and invert to mix thoroughly before transferring the solution into spin column. If too sticky, vortex for 15 seconds. |
| Degraded DNA | Degraded by cellular nuclease in tissue | Please store samples at -20°C before treatment and do not use too much samples. |
| No Product | Did not add ethanol to Buffer WB | Add ethanol before use. |
| Low elution of DNA | Ethanol (Buffer WB ) in spin-column or collecting tube bottom | Perform step 7 according to the instruction. |
| | Use water or other solution replace buffer EB | Perform step 8 according to the instruction. Use Buffer EB |
| $A_{260}$ too high | Eluted silica membrane affects $A_{260}$ value | Centrifuge at 13,000 rpm for 1 minute, carefully harvest the supernatant |
| DNA digestion inhibition | Eluted silica membrane inhibits digestion | Centrifuge at 13,000 rpm for 1 minute, carefully harvest the supernatant |
| | Ethanol in spin-column or collecting tube bottom | Perform step 8 according to the instruction，then air dry in RT |