# CYDN07-CHANGYU Plus DNA Mini Kit (Cells or Tissues)

| Kit Contents | Storage | 50 Preps (CYDN0701) | 100 Preps (CYDN0702) |
|---|---|---|---|
| Buffer TL | RT | 11 ml | 22 ml |
| Buffer CB | RT | 11 ml | 22 ml |
| Buffer IR | RT | 25 ml | 50 ml |
| Buffer WB | RT | 15 ml<br>*Add indicated ethanol before first use* | 25 ml |
| Elution Buffer | RT | 15 ml | 20 ml |
| Proteinase K (20mg / ml) | –20 °C | 20 mg x 1 tube | 20 mg x 2 tubes |
| DNA Bind Columns | RT | 50 | 100 |

**Note:** Proteinase K is a lyophilizate. Centrifuge a few seconds and reconstitute with 1 ml distilled water, aliquot solution. Store at –20 °C**.** Avoid repeated freezing and thawing.

All reagents, when store in indicated temperature, are stable for 9 months.

## Description

Cells are lysed during a short incubation with Proteinase K in the presence of a chaotropic salt (guanidine-HCl), which immediately inactivates all nucleases. Cellular nucleic acids (NA) bind selectively to special silicon membrane pre-packed in the spin columns. Bound NA is purified in a series of rapid "wash-and-spin" steps to remove contaminating cellular components. A special Inhibitor Removal Buffer (Buffer IR) has been included which allows even the application of heparinized sample material with 100 U/ml of Heparin. Finally, low salt elution releases the NA from the silicon membrane. This simple method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

## ❖ Features

1. No toxic phenol or chloroform is used.
2. Organic extractions or ethanol precipitation is not required.
3. Multipurpose and one kit for a variety of sample materials.
4. Optimized protocols allow for fast and easy extraction of DNA.

## ❖ Procedure

**Note：**

⇨ **Before the first use, add the indicated amount of ethanol into Buffer WB bottles, mix well, and mark the bottle with a check.**

**1. For blood with nonnucleated erythrocytes, follow step 1a; for blood with nucleated erythrocytes, follow step 1b; for cultured cells, follow step 1c.**

Blood from mammals contains nonnucleated erythrocytes. Blood from animals such as birds, fish, or frogs contains nucleated erythrocytes.

1a. Nonnucleated: Pipet 20 µl proteinase K into a 1.5 ml microcentrifuge tube. Add 200 µl anticoagulated blood (If blood is less than 200 µl, adjust the volume to 200 µl with PBS. Continue with step 4.

1b. Nucleated: Pipet 20 µl proteinase K into a 1.5 ml microcentrifuge tube. Add 10–20 µl anticoagulated blood. Adjust the volume to 200 µl with PBS. Continue with step 4.

1c. Cultured cells: Centrifuge the appropriate number of cells (maximum 5 x $10^6$) at 13,000 rpm for 10 sec (or 800 rpm for 5 min). Resuspend the pellet in 200 µl PBS. Add 20 µl proteinase K. Continue with step 4.

**Optional:** If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 4.

**2. For animal tissue:**

2a. Cut up to 20-40 mg tissue (up to 10 mg spleen) into small pieces, and place in a 1.5ml microcentrifuge tube. Or pestle it into powders in liquid nitrogen, transfer 20-40mg power into a 1.5ml tube. Add 180 µl Buffer TL and mix well.

2b. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 1-3 hours or until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Continue with step 4.

**Optional:** If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 4.

**3. For mouse tail:**

3a. Cut 0.2–0.5 cm (25-50 mg) mouse tail into small pieces, and place in a 1.5ml microcentrifuge tube. Add 180 µl Buffer TL and mix well.

3b. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C for 3 hours or overnight until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample.

3c. Add 200µl Buffer CB and 100µl isopropanol. Mix thoroughly by vortexing.

3d. Centrifuge at 13,000 rpm for 5 min.

3e. Pipet the supernatant from step 3d into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 1 min. Discard flow-through. Continue with step 7.

4. Add 200 µl Buffer CB. Mix thoroughly by vortexing, and incubate at 70°C for 10 min.
5. Add 100 µl isopropanol to the sample, and mix thoroughly by vortexing. Pipet the mixture from step 5 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 13,000 rpm for 1 min. Discard flow-through.
6. Add 500 µl Buffer IR. Centrifuge at 12,000 rpm for 30 s. Discard the flow-through.
7. Add 600 µl Buffer WB, and centrifuge at 12,000 rpm for 30 s. Discard the flow-through. Repeat Step 8 with another 600 µl Buffer WB.
8. Place the spin column back into the same collection tube. Centrifuge the empty column at 13,000 rpm for 2 min to completely remove ethanol from the column.
   **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.
9. Place the column in a clean 1.5 ml microcentrifuge tube. Add 100 µl of Elution Buffer (Optional: pre-warm the water to 70–90°C will increase the DNA yield) to the center of the column membrane. Incubate at room temperature for 2-3 min, and centrifuge at 12,000 rpm for 1 min to elute the DNA.
   **Note:** Use smaller volume (minimum 30µl) of Elution Buffer will obtain higher concentration.
   **Optional:** Put eluate back to the spin column to repeat elution once. This increases concentration of DNA about 10-15%.

**This product is furnished for LABORATORY RESEARCH USE ONLY.**
**Not for diagnostic or therapeutic use.**