CYDN36-CHANGYU Urine Genomic DNA Kit

Catalog number	Package
CYDN3601	50 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Binding Buffer CB or Inhibitor Removal Solution IR may precipitate at low temperature; redissolve in a 37 °C water bath for a few minutes until clear and cool to room temperature before use. Proteinase K is supplied as a lyophilized powder to preserve activity and facilitate transport; after receipt, centrifuge briefly and dissolve in 1 ml of sterile water; since repeated freeze-thaw cycles may reduce activity, aliquot 20 µl per use immediately after dissolution and store at -20 °C. Keep all solutions tightly capped after use to avoid evaporation, oxidation and pH drift.

Kit components

Component	Storage	50 preps (CYDN3601)
Buffer UB	15-25 °C	10 ml
Binding Buffer CB	15-25 °C	15 ml
Inhibitor Removal Solution IR	15-25 °C	25 ml
Wash Buffer WB	15-25 °C	15 ml (add ethanol before first use)
Elution Buffer EB	15-25 °C	15 ml
Proteinase K powder (optional, 20 mg/ml)	-20 °C	20 mg
Spin columns AC	15-25 °C	50
Collection tubes (2 ml)	15-25 °C	50

Before first use, add the indicated amount of absolute ethanol to Wash Buffer WB and tick the box on the label.

Description

DNA in urine comes from cells shed from the urinary tract. Using urine DNA for basic molecular biology research and clinical diagnosis has several particular advantages: 1) urine collection is non-invasive and atraumatic; 2) extracting DNA from urine is simpler than extracting DNA from blood. This product is designed specifically for extraction of genomic DNA from urine; the extracted DNA can be used directly for PCR. The product has the following features: 1. Simple operation: the whole process takes about 20 minutes at room temperature and is suitable for large-scale sample processing. 2. DNA yield is generally 50-200 ng per ml of urine for women and 3-50 ng per ml of urine for men. 3. The extracted DNA is pure and can be used directly for PCR, DNA methylation analysis, cancer detection and other applications. 4. Safe and non-toxic: the kit is non-toxic to humans and has no corrosive or irritating odor. 5. Cost-effective.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm in a conventional benchtop centrifuge such as an Eppendorf 5415C or similar.

Note: Isopropanol must be supplied by the user.

Note: Preheat the water bath to 70 °C before starting.

❖ Procedure

Before first use, add the indicated amount of absolute ethanol to Wash Buffer WB, mix thoroughly and tick the box on the label.

1. Take 5-50 ml of urine into a centrifuge tube of appropriate size and centrifuge at 3,000 rpm to collect the cell pellet.

2. Carefully discard the supernatant and add 200 µl of Buffer UB to resuspend the cells.

3. Add 20 µl of proteinase K (20 mg/ml) solution, mix thoroughly, then add 200 µl of Binding Buffer CB, vortex immediately to mix thoroughly and place at 70 °C for 10 minutes. The solution should become clear.

4. After cooling, add 100 µl of isopropanol and vortex immediately to mix thoroughly; a flocculent precipitate may appear at this point.

Immediate vortexing or thorough pipetting in the above steps is very important; insufficient mixing severely reduces the yield. If the sample is viscous and difficult to mix, vortex for 15 seconds.

5. Load the mixture from the previous step (including any precipitate) onto spin column AC placed in a collection tube; centrifuge at 13,000 rpm for 30-60 seconds and discard the flow-through.

6. Add 500 µl of Inhibitor Removal Solution IR, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 500 µl of Wash Buffer WB (check first that absolute ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

8. Add 500 µl of Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

9. Return column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

10. Transfer column AC to a clean centrifuge tube. Apply 30 µl of Elution Buffer EB to the centre of the membrane (prewarming the buffer in a 65-70 °C water bath improves the result), leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency. If a higher DNA concentration is required, the elution volume may be reduced, but not below 15 µl; too small a volume reduces DNA elution efficiency and yield.

11. The DNA can be stored at 2-8 °C, or at -20 °C for long-term storage.

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