# Hipro Circulating Cell-Free DNA (cfDNA) Isolation Kit

From human plasma, serum, urine and other body fluid

Cat. #: W2603-20 (20 reactions); W2603-50 (50 reactions)

**Storage**: room temperature.

**Product Description** (This product is for research use only.)

This kit enables efficient isolation of circulating 50 ~ 15,000 bp cell-free DNA (cfDNA) from fresh or frozen **cell-free body fluid**, such as serum, plasma, lymph fluid or urine. This kit use special spin column that can bind circulating DNA, while contaminants pass through. PCR inhibitors, such as salts, proteins and lipids, are removed in wash steps. Pure DNA is then eluted with elution buffer provided in kit. One spin column can process 0.1 ~ 1 mL body fluid sample. The purification is highly reliable and reproducible. The purified DNA can be used for PCR and quantitative real-time PCR and RT-PCR etc.

**Product Components**

| Components | Amount | |
|---|---|---|
| | Cat.#: W2603-20 | Cat.#: W2603-50 |
| Buffer CL | 20 mL | 45 mL |
| Buffer CB (concentrate) | 24 mL | 60 mL |
| Buffer GW1 (concentrate) | 6 mL | 13 mL |
| Buffer GW2 (concentrate) | 6 mL | 15 mL |
| Buffer EB | 6 mL | 15 mL |
| Proteinase K * | 50 mg | 100 mg |
| Proteinase K Storage Buffer | 2.5 mL | 5 mL |
| Spin Column MF | 20 | 50 |
| Collection Tube (2 mL) | 20 | 50 |
| Collection Tube (1.5 mL) | 20 | 50 |

* Store in -20°C after reconstitution. Avoid sit in RT for long time, and avoid repeated freeze-thaw cycles.

**Reagents to Be Supplied by User:** Ethanol (96 ~ 100%), Isopropanol (100%)

**Protocol**

**Important notes and preparation of buffers and reagents**

- **Reconstitute the Proteinase K:** Add Proteinase K Storage Buffer to Proteinase K powder vial (see the vial label for the volume, final concentration is 20 mg/mL), mix well by vortex and inverting until the powder is fully dissolved, and store in -20°C. After reconstitution, the Proteinase K should avoid sitting at room temperature for long time, and avoid repeated freeze-thaw cycles. All other components of the kit can be stored at room temperature (15 ~ 25°C) for up to 1 year. To prolong the lifetime of the kit, store at 2 ~ 8°C.

- Avoid repeated freeze-thaw cycles of the samples. Otherwise, the quality and the quantity of the cfDNA will decrease.
- Maximum 1 mL sample can be processed in each reaction.
- Before use, check if the Buffer CL, Buffer CB has precipitation. If precipitation is found, warm the **Buffer CL, Buffer CB in 56ºC water bath** till all precipitation is re-dissolved.
- Before the first use, add isopropanol (100%) to Buffer CB (see bottle label for the volume), mix well and mark the bottle label.
- Before the first use, add ethanol (96 ~ 100%) to Buffer GW1 and Buffer GW2 (see bottle labels for the volume), mix well and mark the bottle labels.
- If the downstream assay requires RNA-free DNA, add 4 µL DNase-free RNase A (100 mg/mL) to 200 µL sample (serum / plasma etc.). RNase A is not included in this kit. It can be purchased from 101Bio using Cat.# W0601.
- Prepare the water bath to 60°C before experiment.
- Warm up the Buffer EB in 60°C water bath.

**The following is an example of processing 200 µL serum / plasma sample. User can scale up / down the reaction if other volume of body fluid samples are processed.**

1. **Sample preparation:**

   In microcentrifuge tube (not provided) add **200 µL serum / plasma** sample.

   **Note:** if more than 200 µL is processed, scale up the Proteinase K, Buffer CL and Buffer CB proportionally. See table-1.

   **Table-**1:

| Sample volume / Buffers | 200 µL | 300 µL | 600 µL | 800 µL | 1,000 µL |
|---|---|---|---|---|---|
| Buffer CL | 160 µL | 240 µL | 480 µL | 640 µL | 800 µL |
| Buffer CB | 360 µL | 540 µL | 1,080 µL | 1,440 µL | 1,800 µL |
| Proteinase K | 20 µL | 30 µL | 60 µL | 80 µL | 100 µL |

2. Add **20 µL Proteinase K** to the sample, and mix well.
3. Add **160 µL Buffer CL**, invert 15 times, and vortex vigorously for 30 seconds.
4. Incubate at **60ºC for 30 minutes**. Invert to mix several times in the middle.

   **Note:** if more than 200 µL sample is processed, extend the incubation time to 40 minutes.
5. Add **360 µL Buffer CB (check if isopropanol is added)**, invert 15 times, and vortex vigorously for 30 seconds.
6. Sit at **on ice for 5 minutes**. Brief spin to collect all the liquid on the tube wall to the bottom.

7. **Transfer all solution** from step 6 to **Spin Column MF** sit in 2 mL Collection Tube. Centrifuge at **10,000 rpm for 1 minute**. Decant the flow through and place the Spin Column MF back to the 2 mL Collection Tube. If the total volume exceed the capacity of the Spin Column MF, repeat this step.

8. Add **500 µL Buffer GW1 (check if ethanol is added).** Centrifuge at **12,000 rpm for 1 minute**. Decant the flow through and place the Spin Column MF back to the 2 mL Collection Tube.

9. Add **750 µL Buffer GW2 (check if ethanol is added).** Centrifuge at **12,000 rpm for 1 minute**. Decant the flow through and place the Spin Column MF back to the 2 mL Collection Tube.

10. Add **750 µL ethanol (96 ~ 100%).** Centrifuge at **12,000 rpm for 1 minute**. Decant the flow through and place the Spin Column MF back to the 2 mL Collection Tube.

11. Centrifuge at **12,000 rpm for 2 minute**. Decant the flow through and place the Spin Column MF at room temperature for 5 minutes to air dry.

    **Note**: This step is to remove the ethanol residue completely. The leftover ethanol may interfere downstream assays (such as enzyme digestion and PCR etc.).

12. **Transfer the Spin Column MF to a new 1.5 mL Collection Tube, and add 20-100 µL Buffer EB** or $ddH_2O$ to the center of Spin Column MF, sit at room temperature for 2 ~ 5 minutes and **centrifuge at 10,000 rpm for 1 minute**. Collect the flow-through. This is isolated circulating DNA. Use it directly for downstream assay or store at -20°C.

    **Note:**

    1) If downstream assay requires, $ddH_2O$ can be used to elute the DNA.

    2) The PH is important for the elution efficiency. If use $ddH_2O$ to elute the DNA, adjust the PH to 7.0 ~ 8.5 (use NaOH if necessary). The elution efficiency will be low when PH is lower than 7.0.

    3) Before added to the Spin Column MF, pre-heat the Buffer EB to 60°C can increase the yield.

    4) (optional) Use another 20-100 µL Buffer EB or $ddH_2O$ to elute one more time can increase the yield.

    5) (optional) If higher concentration of DNA is needed, use the same the flow through solution to do the elution again, i.e. add the flow through solution back to the Spin Column MF, incubate for 2 to 5 minutes, and centrifuge at 10,000 rpm for 1 minute.

    6) If eluted in $ddH_2O$, the DNA is easy to degrade. Use it immediately. If long-term storage of the eluted DNA is needed, elute in Buffer EB and store at -20°C.

**Customer also buy:**

| DNA Extraction / PCR | Cat. # | Feature |
|---|---|---|
| 1-Drop PCR Mix (squeeze 1 drop do PCR, no pipetting) | W2599-5 | squeeze bottle makes PCR easier |
| Plasmid Miniprep | W0500-50 | 40 % below market price |
| Endotoxin-Free Plasmid Maxiprep | W2104-10 | 40 % below market price |
| Plasmid 96 Miniprep (4 x 96 rxn) | W0506-496 | 50 % below market price |
| 2x Gold Master Mix (with dyes, hot start, HiFi) | W0655-5 | 25 % below market price |

| Virus Packaging | Cat. # | Feature |
|---|---|---|
| Lenti / Retrovirus 10x Titer-Up | P906 / P909 | package 10x more virus |
| Transfection Reagent | P901 | Higher efficiency than lipid-based kits |

| Exosome | Cat. # | 101Bio.com exosome purity | other vendors exosome purity |
|---|---|---|---|
| Exosome Isolation Kit - cell media / serum | P100 / P101 | 95% | 25% ~ 30% |
| Exosomal RNA / Protein Extraction kit | P200 | | |

| Services | Turnaround | 101Bio.com price |
|---|---|---|
| Cloning service | 1 week / step | per request |
| Cell line gene editing - special expertise | 3 ~ 6 months | per request |
| Lentivirus packaging high titer / ultra high | 2 weeks | per request |
| 3rd Generation Aptamer designing service | 3 ~ 6 months | per request |

-- The end --