

## Exosomal DNA Extraction Kit

**Cat. #:** P230-25 (25 reactions); P230-50 (50 reactions)

**Storage:** room temperature. After adding RNase A, store Buffer E1 at 2 ~ 8°C, and it is stable for 6 months. Other buffers and RNase A stock solution can be stored for 12 months at room temperature.

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

This kit is for exosomal DNA extraction from pure exosome isolated by our Exosome Isolation Kits (Cat.#: P100, P101, P120, P121). Normally, 100 ~ 300 ng of exosomal DNA can be purified from the exosome isolated from 200 µL blood serum or 5mL cell media.

The extracted DNA can be directly used for PCR and sequencing. No need to precipitate, concentrate or desalt.

### Product Components

Components	Amount		Storage
	Cat. #: P230-25	Cat. #: P230-50	
Buffer E1	7.5 mL	15 mL	room temperature, 4°C if RNase A is added.
Buffer E2	7.5 mL	15 mL	room temperature
Buffer E3	10 mL	20 mL	room temperature
Buffer PN	5 mL	10 mL	room temperature
Buffer PS	15 mL	30 mL	room temperature
Buffer EB	2.5mL	5mL	room temperature
RNase A ( 10 mg/mL )	42 µL	84 µL	room temperature
PureExo Spin Column	25	50	room temperature
2 mL Collection Tube	25	50	room temperature

### Protocol

**Note:**

- All protocol steps should be carried out at room temperature (15 ~ 25°C)
- Before use, add the provided RNase A to Buffer E1. Store the “RNase added buffer E1” at 4°C.
- All centrifugation steps are performed at **13,000 rpm** in a table-top microcentrifuge at room temperature.

1. Isolate exosomes from 200  $\mu$ L serum or 5 mL cell media using our PureExo Exosome Isolation kit, Cat.# P101 or P100 (not provided). Resuspend exosomes in final volume 100  $\mu$ L PBS .

2. Add **250  $\mu$ L Buffer E1** (check if RNase A has been added) to the 100  $\mu$ L exosomes and mix thoroughly by vortexing or pipetting up and down till no clumps remain.

**Note:** If exosomes are not thoroughly mixed, the DNA yield and purity will be low.

3. Add **250  $\mu$ L Buffer E2** and gently invert the tube 4 ~ 6 times. **Stand for 4 ~ 5 minutes** at room temperature. The solution should become clear and viscous.

**Note:** Do not allow the lysis reaction to proceed for more than 5 minutes.

4. Add **350  $\mu$ L Buffer E3** and mix immediately and thoroughly by inverting the tube 4 ~ 6 times.

**Note:** The white precipitate should form.

5. Sit at room temperature for 5 minutes. Centrifuge for **10 minutes**. Meanwhile place PureExo Spin Columns into a collection tube.

6. Transfer the supernatant obtained in step 5 to **PureExo spin column** (in collection tube)

7. Centrifuge for **30 ~ 60 seconds**. Discard the flow-through.

8. Wash the column once with **200  $\mu$ L Buffer PN**. Centrifuge for **30 ~ 60 seconds**. Discard the flow-through.

9. Wash the column once with **400  $\mu$ L Buffer PS**. Centrifuge for **30 ~ 60 seconds**. Discard the flow-through.

10. Put the spin column back into the collection tube and centrifuge for an additional 2 minutes to remove residual wash buffer.

**Note:** The residual ethanol will affect the subsequent enzymatic reaction (digestion, PCR, etc.). Residual PS buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation.

11. Place the spin column in a clean 1.5 mL microcentrifuge tube (not provided). **Add 10 ~ 300  $\mu$ L Buffer EB (or ddH<sub>2</sub>O)** to the center of the film of each column. Let **stand for 2 minutes** at room temperature and then **centrifuge for 1 minute to collect flow-through**. **The flow-through is purified exosomal DNA**. Use it directly or stored at -20°C.

-- The end --