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EasyMag Exosome DNA Purification Kit

Catalog Number: D036-1, D036-2

Table 1. Kit Components and Storage

Kit Component	D036-1 (25 preps)	D036-2 (50 preps)	Storage	Stability
Buffer AL	8 mL	15 mL	RT	The product is stable for one year when stored as directed.
Buffer DW1*	6 mL	15 mL	RT	
Buffer DW2*	10 mL	15 mL	RT	
Buffer AE	10 mL	15 mL	RT	
MagBinding Beads	1.3 mL	2.5 mL	2-8 ^{o}C	

***** Prior to use, add absolute ethanol to **Buffer DW1** and **Buffer DW2** according to the bottle label.

Product Description

EasyMag Exosome DNA Purification Kit is designed for the isolation of exosomal DNA from pure exosome isolated by our Exosome Isolation Kits (Cat. #: D030, D031, D032, D033, D034). The magnetic beads technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. In addition to easily being adapted with automated systems, this procedure can be also be scaled up or down depending on the amount of starting sample. The extracted DNA is ready for downstream applications such as sequencing, and PCR. No need to precipitate, concentrate or desalt.

Features

- Fast: The purification process takes only 30 minutes.
- Fully compatible: The isolated DNA can be used in most downstream applications such as PCR and sequencing.
- Clean: Minimal carryover of co-precipitating proteins.

Purification Protocol

1. Exosome isolation

   Cell culture media: using our ExoFast™ Exosome Isolation Reagent from cell culture media (Cat. No. D030) to isolate exosome.

   Serum: using our ExoFast™ Exosome Isolation Reagent from serum (Cat. No. D031) to isolate exosome.

   Plasma: using our ExoFast™ Exosome Isolation Reagent from plasma (Cat. No. D032) to isolate exosome.

   Urine: using our ExoFast™ Exosome Isolation Reagent from urine (Cat. No. D033) to isolate exosome.

   Other body fluids (cerebrospinal fluid (CSF), ascitic fluid, amniotic fluid, milk, and saliva): using our ExoFast™ Exosome Isolation Reagent from other body fluids (Cat. No. D034) to isolate exosome.

2. Transfer 250 µL of exosome suspension to a 1.5 mL centrifuge tube. If the volume of the sample is less than 250 µL, bring the volume up to 250 µL with PBS or TE buffer.

3. Add 250 µL Buffer AL to the sample. Vortex to mix well, and incubate at 65^{o}C for 15-30 min.

   Note: Buffer AL may be precipitate during storage, if happen, heat it at 55^{o}C to dissolve. If RNA need be removed, add 10 µL RNase A Solution (10 mg/mL) to the sample.

4. Add 250 µL isopropanol and 50 µL MagBinding Beads to the sample, and vortex for 30 seconds.
5. Mix by shaking, and incubate at RT for 5 min.
6. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
7. Add 600 µL of Buffer DW1 to the tube. Re-suspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

   Note: Buffer DW1 must be diluted with absolute ethanol according to the bottle label before use.

8. Add 600 µL of Buffer DW2 to the tube. Re-suspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

   Note: Buffer DW2 must be diluted with absolute ethanol according to the bottle label before use.

9. Repeat step 8 for a second wash step.
10. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

11. Add 50-100 µL Buffer AE to the tube, resuspend the beads by vortexing for 1 min. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
12. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
13. Store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Poor yield	Incomplete sample lysis • The sample was not mixed well with Buffer AL. After adding Buffer AL, invert and mix for 3-5 times, then vortex at maximum speed to mix the sample with Buffer AL. Insufficient elution buffer volume • Beads pellet must be covered completely with elution buffer and needs to be fully resuspended. Wash buffer no ethanol added • The wash buffer Buffer DW1 and Buffer DW2 must be diluted with absolute ethanol before use.
Low purity	Insufficient washing procedure • Make sure that beads are resusoended completely during the washing procedure.