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EasySC Exosome DNA Purification Kit

Catalog Number: D035-1, D035-2

Table 1. Kit Components and Storage

Kit Component	D035-1 (25 preps)	D035-2 (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	
Mini Column	25	50	RT	
2 mL Collection Tube	25	50	RT	

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

Product Description

EasySC 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 kit also features a buffer system that facilitates complete exosome lysis for efficient nucleic acid isolation. Exosomal DNA is bound to spin column, washed and eluted. Normally, 100-300 ng of exosomal DNA can be purified from the exosome isolated from 200 µL blood serum or 5 mL cell media. 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 to the sample, and vortex for 30 second. Centrifuge briefly to collect any drops from the inside of the lid.
5. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer the samples from step 4 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.
6. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer DW1 must be diluted with absolute ethanol according to the bottle label before use.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer DW2 must be diluted with absolute ethanol according to the bottle label before use.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer DW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.
9. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

   Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
10. Transfer the DNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube, add 30-100 µL Buffer AE preheated to 70ºC. Let sit at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
11. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column is clogged	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. • Add too much sample. Reduce sample amount.
Low purity	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 washing procedure • The wash buffer Buffer DW1 and Buffer DW2 must be diluted with absolute ethanol before use.
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 • Increase elution buffer volume, and repeat elution step. Wash buffer no ethanol added • The wash buffer Buffer DW1 and Buffer DW2 must be diluted with absolute ethanol before use.