DiagExo® Serum Exosomal Protein Extraction kit

Cat. #: P300 **Storage:** keep all bottles upright, at proper temperature (see component table below).

Shelf Life: 6 months

Application: For extraction of **exosomal protein** from human serum, breast milk, saliva, peritoneal fluid, cerebrospinal fluid (CSF), GI fluid, and amniotic fluid etc. This product is for research use only.

Product Size: 10 reactions

Product Description

Our DiagExo® **Serum Exosomal Protein Extraction kit** enables fast and efficient extraction of exosomal proteins from as little as **100 µl serum**. The high yield of exosomal proteins for further downstream applications: ELISA, protein mass spectrometry, protein biomarker verification of exosomal proteins. It also works for similar volume of breast milk, saliva, peritoneal fluid, cerebrospinal fluid, lymph fluid, GI fluid, and amniotic fluid.

Product Contents: (10 reactions)

Component	Amount	Storage
Solution A (orange)	1 mL	room temperature
Solution B	1 mL	room temperature
Solution C *	4 mL	room temperature
DiagExo® Columns	10 x (1.5 mL)	room temperature
DiagExo® lysis buffer	1 mL	-20°C **

* Cap the Solution C bottle immediately after each use. Keep the bottle and handle in dark place.

** Short term (up to 7 days) store at 2–8°C. Long term, aliquot and store at -20°C

Reaction Volume Table (Important)

Suggested volume	Serum	CSF	Amniotic fluid	Inflamma-tory fluid	Lymph fluid	Breast milk	Saliva	GI fluid	Broncho alveolar lavage fluid
Solution A 20 µl Solution B 20 µl Solution C 80 µl	100 µl	100 µl	100 µl	100 µl	100 µl	200 µl	400 µl	400 µl	400 µl
Solution A 40 µl Solution B 40 µl Solution C 160 µl	200 µl	200 µl	200 µl	200 µl	200 µl	400 µl	800 µl	800 µl	800 µl
Solution A 60 µl Solution B 60 µl Solution C 240 µl	300 µl	300 µl	300 µl	300 µl	300 µl	600 µl	1.2 mL	1.2 mL	1.2 mL
Solution A 80 µl Solution B 80 µl Solution C 320 µl	400 µl	400 µl	400 µl	400 µl	400 µl	800 µl	1.6 mL	1.6 mL	1.6 mL
Solution A 100 µl Solution B 100 µl Solution C 400 µl	500 µl	500 µl	500 µl	500 µl	500 µl	1 mL	2 mL	2 mL	2 mL

Protocol (To process 100 µl to 0.5 mL sample of body fluids as listed in the table. **Do not use higher volume** than listed in the table to avoid indistinct layer separation and column clogging.)

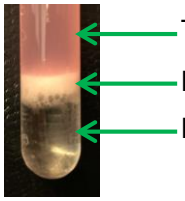
1. Collect body fluid samples, and keep on ice. If you start with frozen samples, thaw them completely at room temperature, and then keep the samples on ice.
2. Centrifuge the sample at **2,000× g** for **10 minutes** at **4°C** to remove cells and debris.
   - ❖ Important: skip this step may cause filter clog in step 14.
3. Without disturbing the pellet, transfer proper volume of **clear supernatant** to a new **glass tube 1**, and keep it on. Please refer to the "Reaction Volume Table" for the volumes of sample volume vs. solution A/B/C.
4. Prepare mixture of A/B/C: In **glass tube 2**, according to the "Reaction Volume Table", add solutions in the **following order**:

1st	Solution A	refer to above table
2nd	Solution B	refer to above table
3rd	Solution C *	refer to above table

* Cap the Solution C bottle immediately after each use.

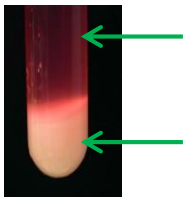
5. **Vortex gently** tube 2 (mixture A/B/C) **5 - 10 seconds** to obtain a homogenous solution.
6. Add **tube 2** (mixture A/B/C) to **tube 1** (body fluid sample).
7. Tightly cap tube 1, vigorously vortex for **30 seconds**, then incubate at **4°C** for **1 hour**.

8a. The mixture now appear as 3 layers:

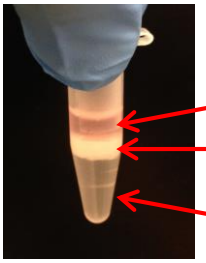


Carefully, aspirate the top layer using a pipette without disturbing the middle fluff layer and discard it. Then go to step 9.

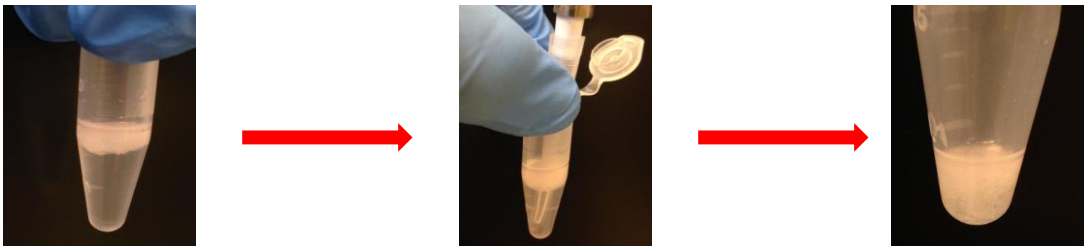
8b. Sometimes, only two layers (orange color top layer and cloudy bottom layer) are visible, remove and discard the top layer. Go to step 9.



9. Transfer the left over in the tube to a new Eppendorf tube and spin at **1,000× g** for **3 minutes**. A new three-layer separation will occur: top orange color layer, middle fluff layer and bottom colorless layer. (See figure on next page for detail.) Proceed to next step within 10 seconds after centrifugation. (The layer separation becomes indistinct 60 seconds after centrifugation.)



10. Pipet out and discard the top layer. Insert pipette tip down to the tube bottom to remove the colorless bottom layer **completely**. Therefore only the "fluff" is left in the tube.



(Top layer removed) (To remove bottom layer) (Only "fluff" layer left in the tube)

11. **Repeat step 9 and step 10 once.**
12. Leave the Eppendorf tube cap open to **air dry for 5-10 minutes** at room temp (do not over dry).
13. Add **1× PBS** as much as 1-2 volumes of the collected fluff to the tube, and resuspend the "fluff" by pipetting up and down **vigorously**.
14. Transfer the suspension carefully into **DiagExo® Column** (provided) and spin the Column at **2,000× g** for **5 minutes** to collect all the flow-through.
15. The "flow-through" is the isolated pure exosome (exosome suspended in PBS). Pipet up and down to resuspend the isolated pure exosome.

Now, you already isolated pure exosome. Use all or partial of the exosome sample to proceed for exosomal protein extraction (next two steps). The left over exosome can be stored in ≤80°C for up to 3 months. Concentrated exosome will precipitate. Re-suspend well before each use.

16. Thaw DiagExo® lysis buffer. Estimate the volume of isolated exosome. Add **an equal volume of lysis buffer** to the exosome sample for exosomal protein extraction. Pipet up and down the mixture for 5 times till mixed thoroughly. Incubate for **15 minutes on ice**.
17. Spin at **4°C**, **14,000x g** for **10 minutes**. The supernatant is the extracted exosomal protein. Carefully transfer it to a clean tube, and store at **-80°C**.

-- The end --