DiagExo® Serum Exosomal Protein Extraction kit

Cat. #: AMS.P300S (2 rxn); AMS.P300 (10 rxn)

Storage: keep all bottles upright. Store DiagExo® Lysis Buffer at -20°C, and store other components at room temperature.

Shelf Life: 6 months

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

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.

Kit Components

Components	Amount		Storage temperature
	Cat.#: **P300S**	Cat.#: **P300**	
Solution A *	0.6 ml	3 ml	Room temperature
Solution B *	0.6 ml	3 ml	Room temperature
Solution C *	0.6 ml	3 ml	Room temperature
Sample Buffer	4 ml	20	Room temperature
DiagExo® Column	2	10	Room temperature
DiagExo® Lysis Buffer	0.2 ml	1 ml	-20°C **

* Cap all solution bottles 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.

- **Do not process more than 0.5 ml serum or more than 2ml other body fluid** for each reaction. Otherwise it will cause indistinct layer separation and column clogging. One DiagExo® column can be used only for one reaction.

Protocol (example of processing **0.1-0.5 ml serum.**)

1 Prepare Sample: **Collect 0.1-0.5 ml serum sample** and keep it on ice. If start with frozen sample, thaw the sample completely at room temperature, and keep on ice.

2 Centrifuge at **3,000× g** for **15 minutes** at 4°C to remove cells and debris.

- Important: skip this step may cause filter clogging in step 15.

3 Transfer **0.1-0.5 ml** clear supernatant to a **15 ml centrifuge tube** without disturbing the pellet. Add **Sample Buffer** to the supernatant to make a total volume of **2 ml diluted sample**, and keep it on ice. (This dilution also works well for other body fluids starting sample volume range between 0.1 - 1 ml.)

- Important: If starting volume of the sample is more than 1ml, add **same volume of Sample Buffer** and keep it in ice. For example, if starting with 1.2 ml saliva, add 1.2 ml Sample Buffer to dilute it. Proceed to Step 4 using this diluted sample. (This dilution works well for starting sample volume range between 1 - 2 ml.)

4 In **another 1.5 ml microcentrifuge tube**, add Solution A/B/C in the following order to prepare 0.9 mL mixture A/B/C (always prepare mixture A/B/C right before use):

1st add Solution A: 0.3 ml **2nd** add Solution B: 0.3 ml **3rd** add Solution C: 0.3 ml

* *Cap all bottles well immediately after each use to prevent evaporation.*

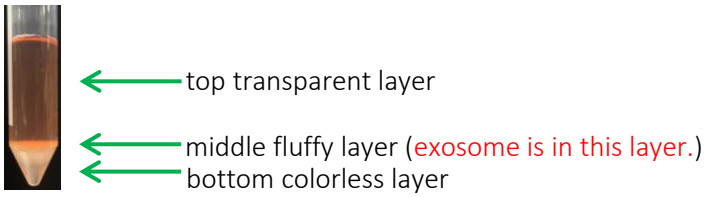
5 **Vortex** the mixture A/B/C for **10 seconds** to obtain a homogenous solution.

6 Add the **0.9 ml mixture A/B/C** to the **2 ml diluted sample** (from step 3).

7 Cap the 15 ml tube, **gently invert the tube for at least 10 times to mix well**, then incubate at **4°C** for **30 minutes**.

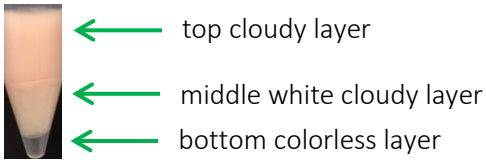
8 Spin the tube at **5,000× g for 3 minutes.**

9 **a.** The mixture now appears as 3 distinct layers:



Do not disturb the middle fluffy layer, and go to Step 10 (refer to Step 9b only if there are not 3 distinct layers).

b. For some samples, layer separation is not distinct. You can imprecisely see 3 layers (as shown in the figure below): top cloudy layer (aqueous layer), middle fluffy layer (thicker and less transparent than top layer) and bottom colorless layer.



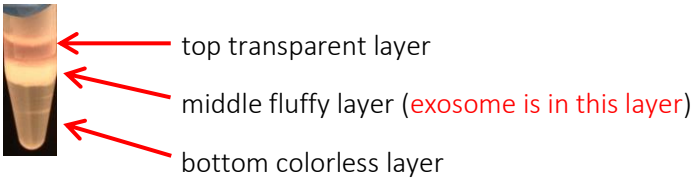
Carefully remove the top layer and discard it. Because the separation is not sharp, be careful, not to disturb or remove the middle fluffy layer because exosome is in this layer.

Prepare **another 0.9 ml mixture A/B/C** as described in Step 4 and 5, and add it to the tube containing the middle fluffy layer and bottom colorless layer. **Gently invert the tube for at least 10 times to mix well**. Incubate at 4°C for another 30 minutes. Spin the tube at **5,000× g for 3 minutes**. Now the mixture appears as 3 layers as shown in Step 9a. Then proceed to Step 10.

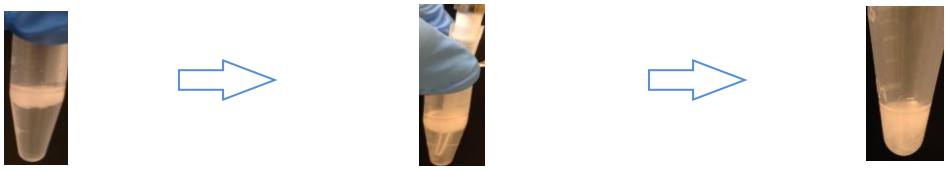
* Extra Solution A, B and C can be purchased separately from 101Bio.com (email to: info@101Bio.com).

10 Pipet out the top transparent layer and discard it without disturbing the middle fluffy layer. Transfer the middle fluffy layer (exosome is in this layer) to **a fresh 1.5 ml microcentrifuge tube**. Spin it at **5,000× g for 3 minutes**. A new 3-layer separation will appear: top transparent layer, middle fluffy layer and bottom colorless layer (see figure below).

❖ Important: Proceed to the next step immediately, without waiting.



11 Pipet out the top transparent layer and discard it. Insert pipette tip down to the tube bottom to completely remove the bottom colorless layer and discard it. Only keep the middle fluffy layer in the tube. (Exosome is in this layer.)



Pipet out the Top transparent layer Remove the Bottom colorless layer **only keep the Middle fluffy layer**

12 Spin again at **5,000x g for 3 minutes,** and 3 layers will appear again. Now, repeat **step 11** for one more time. Now only the **"fluff pellet"** is left in the tube. The **"fluff pellet"** volume is about 25 µl in this example experiment.

13 Leave the tube cap open to **air dry** for 5-10 minutes at room temp (do not over dry).

14 Add **1× PBS** equal to **4 times volumes** of the collected fluff pellet to the tube. In this example experiment, we added 100 µl PBS (4 x 25 µl fluff pellet). Resuspend the fluff pellet by pipetting up and down **vigorously for 40 times**.

15 Shake the tube on a horizontal shaker at high speed for **3 minutes**, and then **pipet up and down vigorously** for 10 times. Repeat this "shake-pipet up and down" procedure for another 2 times.

- **Important:** If the fluff pellet is not re-suspended well, the exosome may be trapped in the fluff pellet resulting in low exosome purity and yield. For some types of samples, eg. Hyperlipidemia patient sample, it is difficult to dissociate the fluff pellet to release exosome. In such cases, extend the pipetting and shaking time in this step.

16 Spin the tube at **5,000x g** for **5 minutes**. Without disturbing the "fluff pellet", transfer the **supernatant** carefully into one **DiagExo® Column** (provided).

- **Important:** Keep the "fluff pellet" at 4°C. Do not discard it until the experiment is finished. See "Trouble shooting" 1.2 for detail.

17 Spin the DiagExo® Column at **3,000× g** for **5 minutes** to collect the "flow-through".

18 The "flow-through" is the **isolated pure exosome** (exosome suspended in PBS).

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.

19 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**.

20 Spin at **4°C**, **14,000x g** for **10 minutes**. The supernatant is the extracted exosomal protein. Carefully transfer it to a clean tube, use if immediately for protein assay or store at **-80°C**. The whole protocol is **completed** here.

Trouble shouting

1. **The final exosome yield is low.**
   1.1. Check if there are left over liquid in the column. If yes, it indicates the column is clogged by contaminated protein. Several reasons could cause the clogging, such as debris was not removed completely in step 2; too much lipid protein in the sample; some precipitation was pipet up in step 15; too much sample was loaded, etc. If this

clogging happens, prepare the sample again, input lower amount of sample, and pay more attention in step 2 and 15.

1.2. For some types of samples, it is difficult to re-suspend the fluff (in step 13 and 14), and the exosome may be trapped in the fluff. Add the final flow-through back to the fluff pellet stored in 4°C (in step 15, a lot of exosomes are trapped in the fluff), pipet up and down **vigorously for 60 times**, and shake the tube on a horizontal shaker for **20 minutes**. **Repeat pipetting** up and down vigorously a few times during the shaking. Go through another column to collect the exosomes.

1.3. For some sample type, the content of exosome is low (the Middle fluffy layer is thin in step 8). Increase the initial input sample volume to collect more exosome.

2. **The flow through has multiple layers.**

There was bottom and/or top layer left in the fluff during step 9-11. Spin the tube at **5,000× g** for **3 minutes**, and carefully pipet out the bottom layer. Pass the sample through a new DiagExo® column to collect the flow-though.

3. **Exosome yield is good, but exosomal protein level is low.**

There may be protein degradation. Always keep the starting material on ice (short term) or at -80°C (within 3 months) before exosome isolation experiment.