Frequently Asked Questions on PureExo kits

Question regarding how to choose our kits

1. Could your kit isolate/purify larger exosome-like vesicles up to 1000 nm in diameter, from culture medium?

   A: No, our kit cannot isolate vesicles bigger than 300nm.

2. Does your P101 kit work for plasma?

   A: It works for both serum and plasma.

3. What's the minimum volume of serum sample can be processed by your PureExo kit (Cat.#: P101)?

   A: The minimum sample volume is 50 µL. We suggest to use 100 µL ~ 400 µL serum or plasma.

4. Can I use your kit to isolate exosome or endothelial microparticles from cultured cells?

   A: Our P100 kit can be used to isolate exosome from cell culture medium. Our P100 kit is not for isolating microparticles from cells.

5. Is your exosome prep contaminated with IgG and/or beads?

   A: Our kits do not use antibody or beads for the isolation. So the final exosome prep is not contaminated with artificial IgG or beads.

6. Do your exosome prep contains albumin?

   A: Some types of cells such as hepatocytes carries enriched albumin, and liver derived exosomes (containing albumin) can be released into the circulation system. So, if the exosomes are isolated from some cell types or serum samples, it may contains albumin.

7. Can I use your PureExo kit (Cat. #P100) on our D.discoideum amoeba?

   A: PureExo exosome isolation kit captures released exosomes or microvesicles from cells. For amoeba exosome isolation, we suggest to collect amoeba culture medium as the same way to collect cell culture medium and then use our kit to do the isolation.

8. What is the composition of the buffer that the final exosomes prep will be in? Will it impact my downstream assay?

   A: The final isolated exosomes are aggregated nano-particles suspended in PBS (containing a very small amount of original culture medium or serum). The suspension buffer is compatible with most of the downstream assays, including RNA/protein extraction, TEM assay, surface labeling, etc.

9. How do I examine that the exosome prep is pure, free of other membrane-derived microparticles (shredding vesicles)?

   A: TEM assay can be used. The isolated microvesicle from cell culture medium or serum using our isolation kits showed sphere membrane encapsulated particles with the diameters varying between 20 ~ 200nm

under EM scanning. These characters help to determine that the harvested microvesicle are exosomes. Shedding particles are reported to be irregular shape with diameter up to 1000nm.

In addition, the size distribution assay by dynamic light scattering can be used. The size distribution pattern of our isolated microvesicles are of diameter between 100 and 200nm.

10. Are exosome functionally active?

A: Function of specific mirRNAs carried by our kits isolated exosomes were examined. Luciferase activity assay showed active function of exosomal microRNA after the exosomes were administrated to cells transfected with targeting mRNA 3'UTR vector.

Questions regarding how to use our kits

11. (P100 and P120 kits) Is it necessary to use glass tubes? And where can I find glass tube?

A: Using glass tubes instead of plastic tubes is required in step 3 and step 4.

Please find glass tubes from FisherScientific. http://www.fishersci.com (Search for Fisherbrand* Disposable Borosilicate Glass Tubes Cat. **NO: 14-961-26**).

12. (P100 and P120 kits) Why can't I see clear fluff middle layer?

A: The following handling errors may lead to the failure:

1). Solution A/B/C did not mix completely before adding the cell culture medium or serum.

2). Did not perform a low speed centrifugation to remove cells and cell debris.

3). The ratio is off. For example: 0.125 mL A + 0.125 mL B + 0.5mL C to 2 mL medium. Overproportion of serum/medium volume to Solution mixture (A/B/C) will lead to an indistinctive interphase.

4). The maximum starting volume is 4 ml medium, and it should be from half million cultured cells or less. If cell number or medium volume exceed this amount, the sample should be splits into multiple samples to proceed.

5). Cultured cells were not starve for 48 hours to remove contaminated exosomes derived from FBS.

13. What biomarker(s) do you use to identify / characterize the isolated exosomes?

A: We use exosome markers such as flotillin, CD9, CD63, CD81, Alix, and TSG101 etc to characterize the isolated exosomes.

14. For downstream qRT-PCR assay, what kits should I choose?

A: We recommend the following kits for the assay:

http://www.qiagen.com/us/products/catalog/assay-technologies/mirna/miscript-ii-rt-kit/

http://www.qiagen.com/us/products/catalog/assay-technologies/mirna/miscript-sybr-green-pcr-kit/#productdetails

15. What internal controls do you recommend for exosomal RNA RT-PCR assay?

A: It depends on the specific sample processed. Please check reference for the controls for your specific exosome samples. The following are reference, again, you have to decide what control(s) to use depending on your specific project.

cel-miR-39-3p
SNORD61
SNORD68
SNORD72

16. What is the yield of exosome using your kits?

A: For P100 kit (PureExo kit for cell culture medium), typically, 50 ~ 100 ul exosomes can be isolated from 2ml cell culture media (Up to 4 ml of medium can be processed per reaction).

If all the harvested exosomes are used for exosomal protein extraction (using our P200 kit), the total protein yield ranges from 150 ~ 200 µg / 2mL medium, 300 ~ 400 µg / 4 ml medium.

If all the isolated exosome are used for RNA extraction (using our P200 kit), total exosomal RNA yield is 50 ~ 100 ng / 2ml medium, 100 ~ 200 ng / 4ml medium.

For P101 kit (PureExo kit for serum or plasma), typically 100 ~ 200 µL exosome (PBS suspension) can be isolated from 100µL to 400µL serum.

If all the harvested exosomes are used for exosomal protein extraction (using our P200 kit), the total exosomal protein yield ranges from 300 ~ 400 µg protein.

If all the isolated exosome are used for RNA extraction (using our P200 kit), total exosomal RNA yield is 200 ~ 300 ng exosomal RNA.

For P120 kit (DiagExo kit for Urine), typically 50 ~ 200 µL exosome can be isolated from 3 mL urine.

If all the harvested exosomes are used for exosomal protein extraction (using our P200 kit), the total exosomal protein yield ranges from 150 ~ 400 µg protein.

If all the isolated exosome are used for RNA extraction (using our P200 kit), total exosomal RNA yield is 50 ~ 200 ng exosomal RNA

Product	To Process	Suggested Processing amount	Expected Yield			Remarks
			Exosome (in PBS)	Exosomal Protein	Exosomal RNA	
P100 PureExo Cell	Cell Media	2 ~ 4 mL	50 ~ 200 µL	150 ~ 400 µg	50 ~ 200 ng	1, the exosomal protein and RNA yield are tested using our P200 kit. If other kits are used, the yield may be lower. 2, the yield per reaction varys on different samples.
P101 PureExo Serum	Serum	200 µL	100 ~ 200 µL	300 ~ 400 µg	200 ~ 300 ng	
P120 DiagExo Urine	Urine	3 mL	50 ~ 200 µL	150 ~ 400 µg	50 ~ 200 ng	
P121 DiagExo BodyFluid	Body Fluid	0.1 ~ 1 mL	vary	vary	vary	
P200 RNA/Protein Kit	Exosome	50 ~ 100 µL	/	150 ~ 200 µg	50 ~ 100 ng	
P300 Serum ExoProtein	Serum	200 µL	100 ~ 200 µL	300 ~ 400 µg	/	

17. (P101 kit) Can I use heparin or EDTA tube to collect blood sample if I need to isolate exosome from plasma?

A: No. Heparin will significantly impair the downstream RNA assays. EDTA may be interfere with downstream PCR assay. Use No-heparin-No-EDTA tube to collect the blood sample. Immediately centrifuge the sample to collect the plasma for exosome isolation. If anticoagulant has to be used, use EDTA tube to collect the blood. Adjust Mg^{++} concentration in the downstream PCR reaction if necessary.

If your question(s) is not answered by this FAQ, please send your questions to support@101Bio.com. Your question will be answered within 24 hours.

2015-5-4