Frequently Asked Questions?

1. What are exosomes?
   Exosomes are extracellular lipid nanovesicles, in the order of 30-120 nm, secreted by various cells and is found in all bodily fluids such as plasma, urine, serum, saliva and cerebrospinal fluid (CSF).
2. What is the difference between exosomes and microvesicles?
   Exosomes are smaller in diameter than microvesicles (30-120 nm vs 100-1000 nm). They have different protein markers and play different roles in cellular communication and genetic exchange.
3. What are the current exosome Isolation products available from BioVision?
   We offer a number of products specifically for exosome isolation. For more detailed information, please go to http://www.biovision.com/exosome-research-3034/exosome-isolation-tools-3039/
4. What is the principle of various exosome isolation products available from BioVision?
   We have a polymer based chemical precipitation (ExoPure™ Reagent), size exclusion chromatography based (ExoPure™ SEC Columns), immunocapture based (ExoPure™ Immunoplates and Immunobeads) and filtration based (ExoPure™ Isolation kits) exosome isolation tools available from BioVision.
5. Could the isolated exosomes be visible to naked eyes from cell media using ExoPure™ Reagent?
   Exosomes are small endosome derived lipid nanoparticles (30-120 nm in diameter), actively secreted by exocytosis in most living cells. It won't be visible by naked eyes. Sometime you might see a visible pellet after the isolation step which you can resuspend in 1X PBS.
6. How did you isolate your plasma exosomes?
   We have various exosome isolation methods for exosome isolation from plasma exosomes including ExoPure™ Reagent, ExoPure™ Size Exclusion Columns, ExoPure™ Immunoplates, ExoPure™ Immunobeads and ExoPure™ Isolation Kits.
7. How to make sure that the exosomes are isolated successfully by your exosome isolation tools?
   You can do a western blot analysis on the isolated, purified exosomes using antibodies to exosomal markers. Just resuspend the purified exosomes in protein sample buffer (without any DTT or β-mercaptoethanol) and then blot for antibodies to exosomal markers such as Alix1 protein, CD9, CD81 or CD63. Additionally, you can do nano tracking analysis (NTA) on the isolated exosomes.
8. What biomarker(s) do you use to identify/characterize the isolated exosomes?
   We use a combination of exosomal markers such as CD9, CD63, CD81, flotillin, Alix1, and TSG101 etc to characterize the isolated exosomes.
9. Is there any specific marker for exosomes isolated from adipocytes?
   It has been shown that almost all adipocyte-derived exosomes express fatty acid binding protein (FABP4).
10. Why specific isolation of exosomes is important?
    If you are interested in selective and specific exosomal marker expressing exosomes, then we recommend immunocapture methods of exosomes isolation i.e using immunobeads or immunoplates. However, same exosomes can express more than one exosomal markers.
11. Do you have exosome isolation reagents from Stem Cell Media?
    Yes, we do have exosome isolation kit for isolating exosomes from stem cell media (Cat# K1239-2, -10).
12. What are the advantages of BioVision exosome isolation tools as compared to ultracentrifugation?
    - Ready to use, easy and fast protocol
    - Time and money saving
    - We can process more than a few samples at a time
    - Small starting sample volume
13. The data sheet says up to 100 µl of starting sample volume. Do you have any information on how much protein or RNA that yields? Also, have you tested with lower or higher sample volumes?
    100 µl is the best volume of fluids to load on ELISA plates. Loading 200 µl does not substantially change the vesicle yield, but increases the background. Loading 50 µl is possible, with a consequent reduction of the signal in ELISA assay. About RNA yield: it is possible to isolate RNA from the vesicles captured onto the immunoplates. But, since the vesicle quantity in each well is low, we recommend collecting the vesicles lysates from at least 4 wells to have a consistent RNA yield.
14. Have you attempted to elute intact exosomes from the ELISA plate?
    We do not provide any tools for eluting exosomes from the ELISA plate. Customer can try making the following buffer (Glycine 10 mM, Triton 0.05%, pH: 2.5). Due to the low yield of vesicles in each well, we recommend collecting together the eluted vesicles for at least 4 wells to proceed with further analyses. But I cannot say for sure if this method works. However, using immunobeads, you can purify exosomes and elute from the immunobeads.
15. How do you isolate and quantify exosomes in one step?
    You can use BioVision's ExoQuant™ ELISA kits for the quantitative and qualitative analysis of exosomes from small amount of human biological fluids (plasma, serum, urine, saliva) or cell media. Transparent and white plates are available depending on the downstream detection approach (colorimetric and luminometric respectively).
16. What are other methods of exosome quantification?
    Other than ExoQuant™ ELISA and ExoQuant™ FACS kits, once can use Electron Microscopy (based on particle size and hence distinguishes between exosomes and other vesicles), Nanosight (optical microscopy based approach to quantify small particles like exosomes) and QPCR analysis for snRNA U6, snoRNA U38B, and snoRNA U43 (quantification based on RNA).
17. What is the minimum volume of starting sample that can be used for exosome isolation?
    The data sheet says up to 100 µl of starting sample volume. The quantity of exosomes could vary between samples. Thus, we always recommend a larger starting amount of sample especially for urine and cell supernatant samples. For urine and cell media, we recommend starting with atleast 1 ml of the sample volume. Concentrate the urine sample or cell supernatants about 10-20 fold using a spin concentrator. Then load 50-100 µl per well in the ELISA plate. Otherwise, for most of the body fluids the minimum volume tested is 50-100 µl per well. We recommend adding 1X PBS if the volume is less than 100 µl.
18. How to isolate and purify exosomes from tissue samples?

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Since exosomes are secreted, we recommend performing short term tissue explant cultures to isolate exosomes from the tissues. The tissue explants in culture will secrete exosomes. You can collect the cell supernatant and isolate exosomes using the protocol similar to the exosome isolation protocol from cell media.

19. Can I freeze purified exosomes after isolation? Does the freezing affect the exosome structure?
    Ideally, exosomes should be processed fresh. Fresh samples are always preferred over frozen samples. However, -80°C C frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple times (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods. The exosomal membrane is different from the cell membrane as it originates from the endosomal membrane and is rich in phospholipids, sphingolipid, ceramides and tetraspanin proteins such as CD63, CD81 and CD9 that enforce the stability of the membrane. However, the freeze-thaw cycles might affect the morphology and in size determination studies including dynamic light scattering (DLS), nano tracking analysis (NTA), bioassays and electron microscopy (EM).
20. Is it necessary to use DMSO in serum sample for studying exosomes?
    DMSO is a cryoprotectant used to preserve the cell membrane from any damage that may be caused by freezing like crystal formation for instance. However, the size of the exosomes and liquid content in it is very small as compared to the cells. Thus, we do not recommend adding DMSO while freezing your serum samples at -80°C.
21. How do you determine the protein concentration in Exosomes?
    Protein concentration in exosomes can be detected by Bradford or BCA methods after lysis of the exosomes. This will give you amount of protein concentration in exosomes in µg/µl.
22. Is there any specific exosome marker?
    Currently, there is no consensus around a general exosome marker. We highly recommend using atleast a couple of exosomal markers to confirm the exosomes including CD9, CD81 and CD63.
23. How to do you prepare exosome samples for western blot analysis?
    We recommend using non-reducing samples for exosome proteins. Thus, there should be any DTT or beta-mercaptoethanol during boiling of your samples before loading onto the PAGE gel. The detection antibodies for western blot analysis targeting tetraspanins prefer non-reducing conditions.
24. Is there any specific marker for apoptotic bodies and exosomes originated from same cells?
    Annexin V, thrombospondin, and C3b are typical markers of apoptotic bodies and can be used to differentiate them from exosomes.
25. How do you isolate exosomal RNA?
    We offer ExoRNA™ Exosome RNA extraction kits for overall exosome immunocapture and RNA extraction as well as for Tumor-derived exosome immunocapture and RNA extraction (Cat# K1220-K1221).
26. Can I get enough exosomal RNA from 10 ml cell culture supernatant for QPCR?
    Exosome yield depends on the cell confluence, cell type, health of the cells and time of harvest. Using our ExoPure™ tools, once can purify ample amount of RNA for QPCR analysis.
27. What are the negative control for exosome isolation?
    The below mentioned proteins should be absent from the Exosome preparation
    http://www.journalofextracellµlarvesicles.net/index.php/jev/article/view/26913
    Endoplasmic reticulum; Grp94HSP90B1; calnexin (CANX); Golgi (GM130); Mitochondria (cytochrome CCYC1); Nucleus (histonesHIST*H*); Argonaute/RISC complex (AGO*).
28. How do you prepare samples for studying exosomes in cell media?
    Incubate the cells in exosome depleted FBS or incomplete media (without FBS) at 70-80% confluence for 24 hr before collecting the cell media. Then follow the procedure as mentioned in the individual datasheets of the exosome isolation products.
29. BioVision offers two different size of immunobeads for one kind of sample, 0.4 and 1.0 micron? How to choose the size of the beads to isolate one kind of sample, for example from biofluids?
    Yes, we do offer two different sizes of the Immunobeads: 0.4 and 1 micron. If your sample volume is low, we recommend using the 0.4 micron beads. This is because if you use 0.4 micron beads, the number of beads added per volume is more and the total surface for 0.4 micron is bigger than 1.0 micron. They capture better from small volume of samples. Otherwise, if the sample volume is high, the larger size of the beads (0.1 micron) enables more exosome capture per volume added as compared to the smaller size of the beads.
    The bead size is important especially for exosomes present in low numbers in a large volume of the sample. The larger bead provides increased surface area and thus more efficient capture of the rare exosomes.
30. Quantification of exosomes using standard FACS instruments is not possible due to the small size of exosomes: How does your ExoQuant™ FACS quantification system works?
    After Exosome isolation, the purified exosomes are bound to the 4 µm aldehyde sulfate latex beads (FACS-Beads) followed by the exosome marker characterization via FACS analysis using exosomes specific antibodies.
31. Exosome characterization using ELISA can be targeted on external protein epitopes on the vesicle. What is the sensitivity of your ELISA assays and how do you recognize the internal proteins?
    ExoQuant™ ELISA kits are double sandwich ELISA kits that consists of ELISA plates pre-coated with proprietary exosome tetraspanin antibodies thereby enabling specific capture of the external epitopes using different biological samples. The detection limit of an ELISA assay is lower than 0.35 µg of overall exosome which is an equivalent of less than 50 pg of targeted exosomes protein. Additionally, detection of external epitopes can be also done by FACS analysis.
    For internal proteins, we recommend western blot analysis. You can also detect internal proteins via FACS analysis after you permeabilize the exosomes.
32. What are Plasma Exosome Standards?
    Plasma exosome standards are purified lyophilized exosomes isolated from a pool of healthy certified donors by differential ultracentrifugation and microfiltration.
33. For exosome isolation from cell media (using immunobeads), I noticed use of spin concentrators are recommended to concentrate the cell media samples from 10 ml to 1 ml. Do you have any recommendation for the spin concentrators to use?

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You can use the spin concentrators available from any companies with 3K and 10K cutoff.

34. Which antibody you use for the neural exosome isolation plates?
    For proprietary reasons, we cannot reveal the name of the antibody used. We have chosen a marker of neuroblastoma that is well expressed on the vesicle surface that was able to bind the vesicles in ELISA plate and is useful for enriching exosomes from neural origin from those physiologically produced from healthy cells in the body.
35. Regarding the lyophilized exosome standards, do you also the characterize their DNA/RNA contents too in addition to the particle number and the protein content?
    When we produce a new exosome standard lot, we do not test it for nucleic acid extraction, but we have made many tests in the past. In fact, they are used as positive control in RNA kit for exosome RNA isolation. In addition, some customers are using as mutation control in spike-in experiments. Lyophilized standards are currently used as a positive control in RNA and DNA kit, so it is possible to use them for isolation of nucleic acids.
36. Can we detect DNA from the Exosome Standards?
    Vesicles usually contains a very small amount of DNA, the most of it is fragmented and single stranded, and the quantity is not enough for an agarose gel analysis. ExoDNA™ kit can be used for the analysis by QPCR of gene mutations carried by vesicles or circulating in body fluids.
37. Is there any possibility of genomic DNA or mitochondrial DNA (mtDNA) contamination using ExoDNA™ Extraction Kits?
    ExoDNA™ Extraction Kits allows the isolation of circulating+extracellular vesicle (EV)-associated DNA and in EVs do contain genomic DNA. It is also possible that there are also some mitochondrial DNA is present in EVs, but the evidences about this issue are not still very clear. If customers want to limit their analysis only to the DNA contained in EVs, they can perform the DNAse treatment after the precipitation of vesicles+circulating DNA, before to add the lysis buffer. Rather, vesicles protect the DNA from the DNase treatment, which eliminates only the circulating DNA fraction.
38. Is there any possibility of ribosomal RNA contamination using ExoDNA™ Extraction Kits?
    Yes, there is a possibility of RNA contamination, since the vesicles contain RNA, in particular miRNAs. Percentage of ribosomal RNA is very small in vesicles.
39. Does ExoDNA™ Extraction Kits adopt RNAse treatment step?
    No, the ExoDNA™ Extraction Kits does not adopt any RNAse treatment, but it is possible to do the RNAse treatment once the DNA is eluted.
40. For the BioVision FACS kits, CD9 or CD63 antibody is used. Are these FITC conjugated or what is the dye the antibodies are conjugated with?
    In BioVision FACS™ assay kits, antibodies are not conjugated. We provide a secondary antibody Alexa 488 (included in the kit).
41. Do the exosome isolation reagents precipitate apoptotic bodies?
    Apoptotic bodies are much larger (>800 nm), so they will be mostly removed from the sample during pre-spin (along with cells and debris). The exosome reagents are added in the next step, and it precipitates primarily exosomes (30-150 nm).
42. What is the composition of the buffer that the final exosomes preparation will be in? Will it impact my downstream assays?
    The purified exosome pellet is finally suspended in 1X PBS, which is compatible with most of the downstream assays including RNA/protein extraction, QRT PCR, TEM assay, western blotting, surface labeling, etc.
43. Can I use heparin or EDTA tube to collect blood sample if I need to isolate exosome from plasma?
    No. Heparin will significantly impair the downstream RNA assays. EDTA may interfere with the downstream PCR assay. Please 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^{+2} concentration in the downstream PCR reaction if necessary.
44. Can we block/inhibit the release of exosomes from cancer cells?
    Dimethyl ameloride (DMA), Sphingomyelinase imhibitor (GW4869), and Glucosyl ceramide synthase inhibitor (di threo-1-1phenyl-2-de canoylamino-3-morpholino-1-propanol) have been shown to inhibit the release of exosomes in different cell lines. Alternatively, shRNA knock down to Rab27 or Rab35 would be the ideal candidates for inhibiting exosome release.
45. Can you recommend a miRNA isolation and RTs kit for qPCR analysis?
    We offer ExoRNA™ Exosome RNA extraction kit. The kit contains immunobeads for overall exosomes capture, all the solutions, columns and elution tubes for RNA extraction. This kit allows high yield of total RNA extraction including small RNAs, miRNAs.
46. Why is the exosomal miRNA levels higher in plasma as compared to the serum exosomal miRNA?
    It is possible that you are losing exosomes and thus the exosomal miRNA during the removal of the clot from the whole blood during serum sample preparation as compared to plasma where there is less chance of losing vesicles and thus the exosomal miRNA.
47. Does cell death affect exosomes?
    Yes, cell death definitely affects exosomes secretion. Thus, we always recommend healthy and viable cells for studying exosomes. Additionally, apoptotic cells release a lot of apoptotic vesicles.
48. What should be the exosome yield per cell?
    The exosome yields per cell or the total number may depend on several factors, including the cell type, cell confluence, the culture conditions (growth factors etc), the time of harvest, the uniformity in size of exosomes, and the source type. Thus, one cannot fix a number of exosome yield per cell.
49. How do I visualize apoptotic bodies, microvesicles and exosome pellet from centrifugation?
    After centrifugation, you can't distinguish an exosomes pellet from an apoptotic bodies pellet. To characterize individual extracellular vesicles, you can use western blots (WB), flow cytometry (FACS), global proteomic analysis including mass spectrometry techniques or Electron microscopy and Atomic force microscopy for characterizing individual EVs. For larger vesicles including apoptotic bodies, Cytospins and Immunofluorescent images can be used and for size distribution, Nanosight Analysis, Dynamic Light Scattering can be used.
50. What is the best storage condition of conditioned media (at 4°C/-20°C/-80°C) for subsequent isolation of exosomes?
    We recommend processing fresh samples, but cell media can be stored at 4°C for up to one week with no major changes with exosomes/EV being observed. For long term storage, -80°C is recommended.

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51. How much RNA concentration do you expect from serum exosomes?
    Most exosomes carry very less amount of nucleic acids. The concentration of RNA in serum samples is too low to be seen in agarose gel or even by nanodrop. You can try using the RNA Pico Agilent Bioanalyzer chip to check the RNA concentration.
52. Is exosome secreted by quiescent cells?
    Exosomes are formed both during the recycling of the plasma membrane or during intercellular communication. So, it makes sense to say that exosomes are secreted by quiescent cells also.
53. How can I normalize mRNA expression in exosomes?
    You can use miRNA genes such as miRNA16, miRNA26a, miRNA221, miRNA22, miRNA181a, miRNA181c, miRNA103, miRNA191, let7d and small RNAs (5SrRNA and U6snRNA) that is normally expressed in exosomes.
54. How can I fix my exosomes for microscopy after isolation?
    Exosomes are so small that it is difficult to get enough antibody binding to detect them via immunofluorescence. However, transmission electron microscopy have been performed on exosomes.
55. Which antibody is coated on the immunobeads? Is there anything left on the surface of exosome after being eluted from the beads? If there are some chemical group left on the surface of the exosome, whether would they interfere the downstream experiments?
    We have used many different antibodies for coating the immunobeads based on the sample to be used for exosome isolation. For example, if you want to isolate exosomes from biofluids (serum, plasma, urine), we have CD9 & CD63 coated immunobeads. But for immunobeads for isolating exosomes from Cell Media, we have used a different antibody for coating the immunobeads.

    The immunobeads have antibody (s) attached to it. This antibody (on the beads) binds to the antigens on the exosome surface. So, there is no chemical modification on the exosome surface itself. It is just an Antibody (on immunobeads)-Antigen binding (on exosome surface). Thus, using the elution buffer (provided in the kit), the customer can elute the exosomes from the immunobeads. The purified exosomes can be then used for any downstream experiment without any interference.
56. Why immunobeads are used and not ExoPure™ Reagent in the Exosome Immunocapture and RNA Extraction Kit in BV Cat#K1220? Will the immunobeads capture all of the exosomes from the samples?
    In the RNA Extraction Kit (Cat# K1220), we have used immunobeads to capture the overall exosome population from a wide range of samples including cell culture supernatants and human biofluids (plasma, serum, urine, etc.) as well as enrich some specific exosome subpopulations (tumor-derived exosomes). However, since this method is immunocapture based, it will not be able to capture all the exosomes that do not express not expressing the corresponding marker.

    If you want, you can use the ExoPure™ Reagent for exosome purification in the first step (instead of using the immunocapture based exosome isolation). Then use the lysis buffer and spin columns (provided in the RNA Kit, Cat# K1222) for the RNA isolation step.
57. Whether the ExoPure™ Isolation reagent (if used) will interfere with the downstream experiment following exosome RNA extraction?
    ExoPure™ Isolation reagent isolates exosomes in one single step from a small volume of sample. Yes, you can use Isolation Reagent followed by RNA Extraction from the purified exosomes. The Isolation Reagent will NOT interfere with the downstream experiments following exosome RNA extraction. However, we recommend using the more specific, immunocapture method of exosome isolation before the RNA purification.

    Additionally, if you are using the ExoPure™ Isolation reagent for the purification of exosomes, we recommend washing away the Isolation Reagent before the RNA extraction step using Cat# K1222.
58. How much RNA could be extracted using Exosome Immunocapture and RNA Extraction Kit (Cat#K1220)? The customer wants to know the quality.
    As shown in the datasheet for Cat# K1220, we compared our exosome RNA quality following exosome isolation and RNA extraction with our Competitors Kit. We guarantee the quality and yield of our RNA.
59. How much exosome would the exosome ELISA kit detect in each test-well? What's the sensitivity of the exosome ELISA kit for cell media samples?
    The sensitivity of our ExoQuant™ ELISA Kits (Cat# K1205, Colorimetric) test is around 39 pg of protein equivalent. We have shown in our datasheet that 10 µg of lyophilized exosomes are equivalent to 0.1 ng of recombinant exosomal protein. Since the lower concentration in the standard curves is 0.39 µg of lyophilized exosomes and hence the sensitivity of our test is around 39 pg of protein equivalent.

    The number of exosomes in sample also depends on the sample type, cell culture conditions, treatment type, diseased conditions etc.
60. Could this step of centrifugation for 30 min at 10,000g be replaced by other methods because the customer has no this kind of high centrifugal force instrument?
    The customer can also centrifuge at 6000g for atleast an hour instead of 10,000g for 30 min.
61. Do you have products for Extracellular Vesicle (EV) tracking Studies?
    Biovision offers highly purified Fluorescent Exosome Standards for EV tracking in fluorescence microscopy experiments. Fluorescent exosomes are labeled with green dye, which provides a stable fluorescent labeling and exosomes are suitable for different applications providing a long-lasting well visible signal. Fluorescent exosomes are available human biological fluids and cell culture media.