Exosome Precipitation Solution

	REF.	SAMPLE	VOL
Exosome precipitation	EPStep	Cell culture media, Urine	12 ml
Exosome precipitation from Plasma and Serum	EPStep-PS	Plasma, serum	5 ml
Exosome precipitation from Plasma + Thrombin	EPStep-T	Plasma	5 ml

1. PRODUCT DESCRIPTION

Exosomes are small extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB)1, with the plasma membrane. They are thought to provide a means of intercellular communication and of transmission of macromolecules between cells allowing the spread of proteins, lipids, mRNA, miRNA and DNA and as contributing factors in the development of several diseases.

The biological characterization of exosomes requires in most cases the isolation of intact exosomes. In this sense, a large number of methods have been developed for the isolation of exosomes from biological fluids, among which are ultracentrifugation, chromatography, filtration, immunological separation and polymer-based precipitation2. Each one of these methods presents its advantages and disadvantages, being the duration of the method, the need to have specialized equipment, the volume of sample, the purity and the low recovery, some of the disadvantages that these methods present.

The solution of precipitation of Exosomes (EPS) is framed within the methods of precipitation of exosomes based on polymers, and among its main advantages it stands out its simplicity, speed, reproducibility, the slight effect in isolated exosomes and the use of neutral pH. And thanks to its specific formulation, it limits some of the disadvantages of this type of methods such as the co-isolation of non-vesicular contaminants, including lipoproteins and the presence of polymer material that might not be compatible with the downstream analysis.

- **Tested application**: BCA, WB, NTA, qPCR and Flow Cytometry
- **Species reactivity:** Human
- **Recommended usage**: Immunostep's Exosome Precipitation Solution, is intended for the extracellular vesicles (EVs) and specifically exosomes (~50-150 nm) from cell culture media and biofluids (plasma, serum, urine).
- **Presentation:** liquid

2. APPROPIATE STORAGE

Precipitation solution is stored refrigerated between 2 °C and 8 °C.
Thrombin solution should be stored at -20 °C in order to preserve the activity. The kit is stable until the expiry date stated on the vial label if kept at recommended conditions. Do not use after the date indicated.

3. REAGENTS PROVIDED

DESCRIPTION	COMPONENTS	VOL	STORAGE
Exosome precipitation	Exosome precipitation reagent	12 ml	2-8°C
Exosome precipitation from Plasma and Serum	Exosome precipitation reagent	5 ml	2-8°C
Exosome precipitation from Plasma + Thrombin	Exosome precipitation reagent	5 ml	2-8°C
	Thrombin	400 µl	-20°C

4. RECOMMENDATIONS AND WARNINGS

Plasma contains fibrin which can precipitate together with the precipitation solution giving rise to an insoluble pellet, so it is recommended to treat the plasma sample before the precipitation protocol to dissolve the fibrin, increasing exosomes recovery.

5. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

6. SAMPLE PREPARATION

1. Centrifuge the sample (cell culture media, urine, serum, plasma) at 3000xg for 15 at room temperature (RT), to remove cells and debris.
2. Transfer the supernatant to a new tube, taking care of not disturbing the pellet, and place it on ice until needed.

- **Plasma sample De-fibrination.**

Treatment of plasma with thrombin is recommended to convert fibrinogen to fibrin and be easily removed by a simple centrifugation step; if not the exosomes recovery could be affected.

1. Add the adequate volume of thrombin to the centrifuged plasma, to leave the samples at a final concentration of 10U/ml. For example, for 0.5 ml starting volume of plasma add 10 µl of stock thrombin [500 U/ml].
2. Incubate at RT for 5 minutes while mixing by vortexing, or flicking the tube.
3. After incubation, centrifuge in at 10,000xg for 5 minutes at RT.
4. Transfer the supernatant by aspiration to a new tube, taking care of not disturbing the pellet at the bottom of the tube which contains the fibrin. Continue to protocol.

7. PROTOCOL

1. Transfer the required volume of prepared sample to a new tube and add the appropriate volume of Exosome Precipitation Solution, according the Table below.

TYPE OF SAMPLE	SAMPLE VOL	PRECIPITATION REAGENT	INCUBATION TIME
Cell culture media /Urine	5 ml / 10 ml	1,2 ml / 2,4 ml	Overnight
Plasma /Serum	250 µl / 500 µl	63 µl / 126 µl	30 min

2. Mix the sample/solution mixture well by vortexing, pipetting up and down or flicking the tube.
3. Incubate the sample without stirring at 2-8°C overnight for cell culture media and urine, or 30 min for plasma and serum (Table above).
4. After incubation, centrifuge the sample at 1500xg for 30 min at RT.
5. Discard the supernatant by aspiration taking care of not disturbing the pellet, since exosomes are concentrated in the pellet. It is important to remove all the supernatant, so if necessary repeat centrifugation to remove any remaining of the precipitation solution.
6. Resuspend pellet in 100-500 µl of 1X PBS or similar buffer (according your downstream process), by vortexing or pipet up and down. This step can take a few minutes.
7. If you are not going to use the precipitation exosomes immediately, store them at 2-8°C for up to a week or at -20°C for long-term periods.

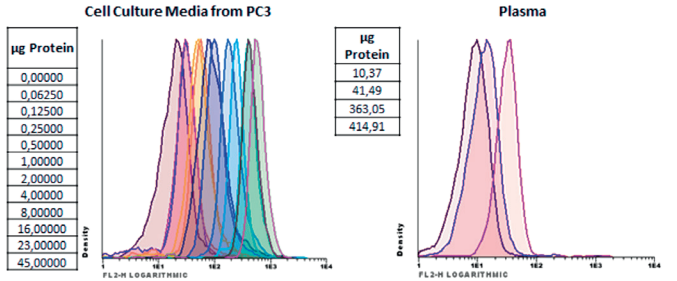
If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services: tech@immunostep.com.

8. PERFORMANCE EXAMPLES

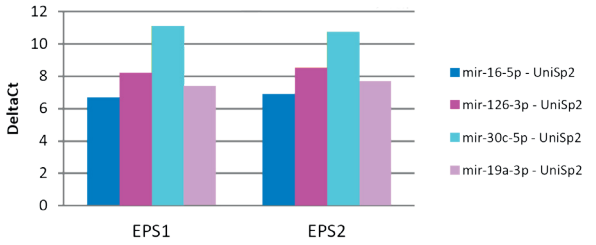
a. **Bradford Protein Assay.** Determination of protein concentration after purification of exosomes from different samples (cell culture medium and plasma), with precipitation solution.

	Cell Culture Media from PC3	Plasma
Cell Culture Media Starting Vol. (ml)	160,00	0,25
Concentration (µg/ml)	1165,04	10372,80
Precipitate Vol. (ml)	0,25	0,25
Amount (µg)	291,26	3554,00

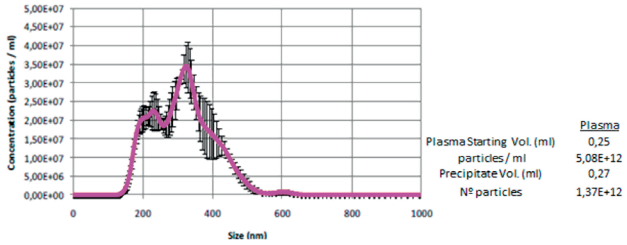
b. **Flow cytometric analysis.** Histogram of exosomes isolated by Exosome Precipitation Solution from cell culture media and Plasma. Captured with anti-human CD63 antibody (Clone TEA3/18) coated beads and detected with biotin anti-human CD9 antibody (Clone VJ1/20) stained with Streptavidin-PE (ExoStep kit Components, Ref, Each peak (histogram) corresponds to a different exosome quantity.



c. **Analysis in duplicate of the exosomal miRNA levels by quantitative RT-PCR.** We followed the instructions in the QIAGEN Handbook to extract miRNAs, do the RT-PCR to obtain the cDNA and then the qPCR or quantitative PCR. Precipitation solution does not interfere with the extraction of RNAs, nor with RT-PCR and allows the detection of endogenous miRNAs miR-126-3p, miR-30c-5p, miR-19a-3p and miR16-5p present in the sample.



d. **NanoSight analysis of the exosomes recovered from Plasma with Total Exosome Precipitation Solution.** EVs recovered with Precipitation Reagent (from Plasma) have a similar size distribution than those isolated by other methods such as ultracentrifugation.



9. REFERENCES

1. Yáñez-Mó M, Siljander P, Andreu Z, Bedina Zavec A, Borràs F, Buzas E et al. Biological properties of extracellular vesicles and their physiological functions. Journal of Extracellular Vesicles. 2015;4(1):27066.
2. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. Current Protocols in Cell Biology. 2006.

10. MANUFACTURED BY

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