PRODUCT INFORMATION & MANUAL

Immunoplates for Monocytes/Platelet-derived Exosome Isolation (Plasma, Luminometric)

NBP2-49823

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both the amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

Novus immunoplates are 96 multi-well plates covalently pre-coated with a proprietary exosome capturing antibody thereby allowing enrichment and selection of exosomes from tumor origin from human plasma. Covalent coating improves the correct orientation of antibodies maximizing the quantity of immunocaptured exosomes and increasing the binding efficiency of the plate. In addition, the coating chemistry reduces the non-specific binding of circulating protein complexes and cell debris, and it helps the enrichment of exosome subpopulations. Novus offers different types of plates for capturing the overall or enriching specific exosome subpopulations (tumor, neural, glial, monocytes and platelets). Plates are blocked and stabilized for long term storage. Plates are pre-coated with anti-Rabbit or anti-Mouse antibodies. The type of antibody used for coating is indicated on the label of the plate. To avoid cross-reactivity in detection, do not use the same type of antibody used for coating. Transparent, white and black plates are available depending on the downstream detection approach (colorimetric, luminometric and fluorimetric respectively). ELISA immunoplates are ready to use.

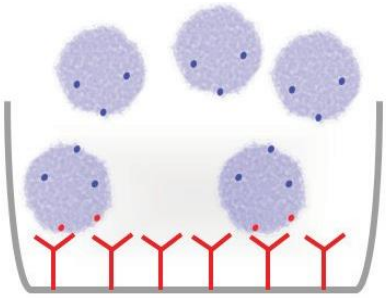

Figure 1. Immunoplates for Monocytes/Platelet-derived Exosome Isolation. Plates are coated with antibodies overexpressed in particular pathological conditions on exosome surface.

II. Application:

- Immunoplates for Monocytes/Platelet-derived Exosome Isolation allow multiple profiling of exosome markers from a single sample or screening of a large number of samples.
- Capture and enrichment of platelet-derived exosomes. Unfractionated plasma sample can be directly used for capture.
- Titration of purified exosomes.
- Immunoplates are also suitable for nucleic acid extraction from immunocaptured exosomes on the plate.
- Small amount of biological sample required (100 µl per well). Open platform for customized coating solutions.
- Immunoplates are ready to use and stable for long term (up to 2 years). No exosome pre-purification is required (by ultracentrifugation or other methods).

III. Sample Type:

- Human biological fluids: Plasma.
- Up to 100 µl of biological sample can be loaded per well.
- Unfractionated plasma sample can be directly used for capture.
- Plates are pre-coated with polyclonal anti-Mouse antibodies.

IV. Package Contents (for enrichment of Monocytes/Platelet-derived exosome):

Components	NBP2-49823
Standard 96-well format pre-coated plate (White plate)	1 plate

V. User Supplied Reagents and Equipment:

- Single-use and/or pipettes with disposable tips 2-100 µl.

VI. Shipment and Storage:

- Immunoplates are shipped and stored at 4°C for up to 24 months, if unopened and 6 months at 4°C after opening. DO NOT FREEZE!

VII. Reagent Preparation and Storage Conditions:

- Immunoplates are individually sealed in an opaque aluminum zip lock bag, compliant to pharmaceutical regulations. The pouch is easy to open and is resealable by zip-lock feature.
- Immunoplates can be stored at 4°C for up to 24 months, if unopened and 6 months at 4°C after opening.

VIII. Immunoplates for Monocytes/Platelet-derived Exosome Isolation (Plasma, Luminometric) Protocol:

1. **Human Plasma sample preparation:** Prepare samples by 3 centrifugation steps to eliminate red blood cells and cellular debris. After each step, transfer the supernatant to a new tube and discard the pellet.
    a) 10 min at 300g at 4°C (save the supernatant; discard pellet).
    b) 20 min at 1200g at 4°C (save the supernatant; discard pellet).
    c) 30 min at 10,000g at 4°C (save the supernatant; discard pellet). Plasma can be diluted 1/1 in 1X PBS.

2. **Exosome binding on ELISA immunoplate: Note: Make sure to never touch the bottom or sides of the wells or you will scrape off your samples/standards. As a reminder "No Touch" is placed on that line.**
    a) The plate is ready to use; no pre-washing is required.
    b) Add up to 100 µl of sample per well, adjust with 1X PBS up to 100 µl if necessary.
    c) Seal the plate with parafilm and incubate at room temperature while shaking for 20 min.
    d) Depending on your sample, incubate overnight: Plasma samples at 4°C.
    e) Wash the plate (washing buffer suggested: PBS + 0.05 % Tween20). Add 200 µl/well of Washing Buffer and discard plate contents by pouring out. No Touch. Wash 3 times with 300 µl/well of Washing Buffer. After each addition, pour off wash. No Touch.

3. Thus, prepared plate can be used for analysis of exosome markers via ELISA assay. White plates are used for the downstream luminometric detection approach following incubation with a primary antibody against an exosome surface marker. Moreover, captured exosomes are suitable for lysis and extraction of exosome-associated RNAs.

4. **Sensitivity:** Immunoplates allow exosome protein profiling without pre-purification (by ultracentrifuge, chemical precipitation or microfiltration). Immunoplates are useful tools for immunocapturing exosomes from biofluids or culture media, for protein analyses and protein marker profiling. They allow quantitative and qualitative simultaneous analysis of different protein markers (Figure 2) from the same sample, or expression profiling of a single marker in different samples (Figure 3), without exosome pre-purification via ultracentrifuge or other methods. CD9 expression (Figure 4) of immunocaptured plasma exosomes mimic that of the corrrespondent ultracentrifuged fraction. No significant cross-reactivity is observed with soluble antigens or other vesicle- associated proteins (Figure 5). These immunoplates are precoated with exosome associated antibody undicative of monocyte/platelet origin enabling specific enrichment of exosomes from human plasma. In the examples reported (Figure 6), we show the specificity of binding of exosome derived from neuroblastoma (SH) or glioblastoma (U87) cell lines when plates for neural or glial-derived exosome capture were used. In Figure 7, increasing amount of purified exosomes from glioblastoma (U87) cell lines were spiked in human plasma from healthy donors. Selective capture of specific exosome subpopulation was tested comparing the same plasma sample after spiking of purified plasma exosomes (HD). In the experiment (Figure 7), the signal relative to the specific exosomes subpopulation increases after spiking, while no changes are detectable with HD, suggesting the enrichment of glial-derived exosomes.

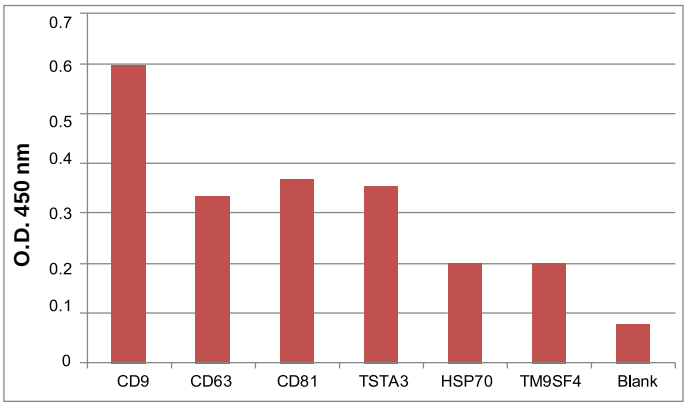

Figure 2. Common exosomal biomarkers analysis in a healthy donor's plasma sample.

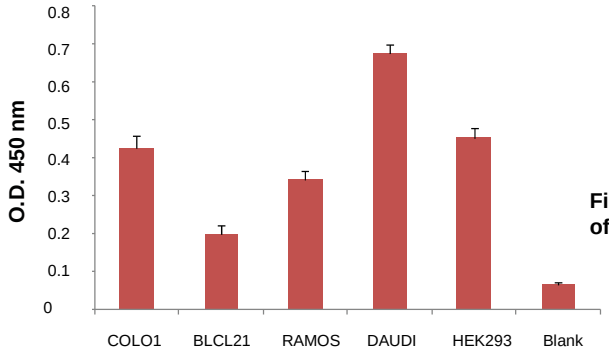

Figure 3. CD63 profiling on exosomes derived from supernatants of different cell lines.

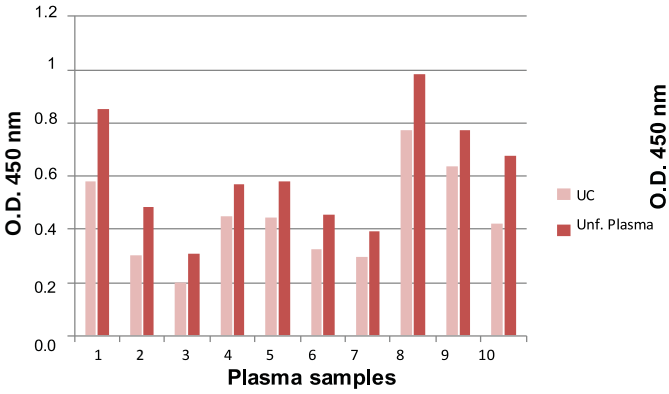

Figure 4. Comparison of CD9 detection on purified (ultracentrifuged) plasma exosomes vs unfractionated samples in a set of healthy donor's plasma.

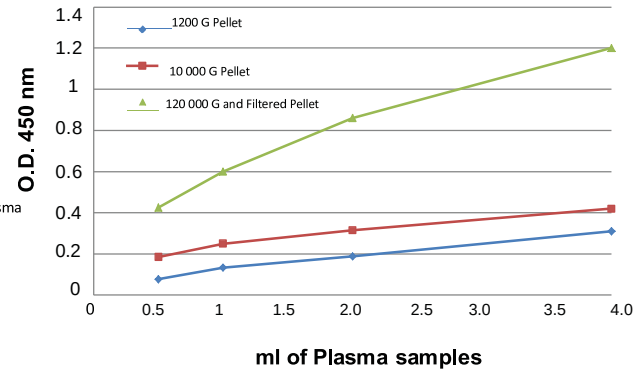

Figure 5. Immunoplate is selective in capturing purified exosomes (pellet after centrifugation 120,000g) and no other circulating microvesicle (pellet 1200g and 10,000g).

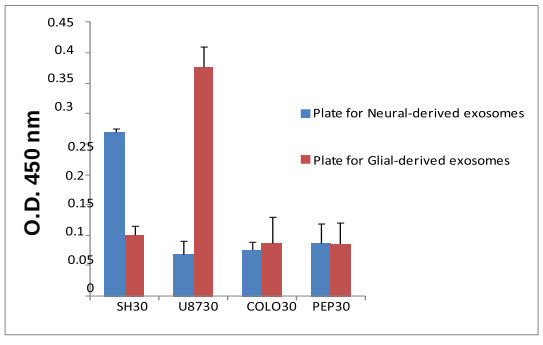

Figure 6. Specific immunocapture of 30 µg of exosome isolated from Neuroblastoma (SH-30) or Glioblastoma (U87-30) cell lines in specific immunoplates. COLO cell and plasma (PEP) purified exosomes were used as controls.

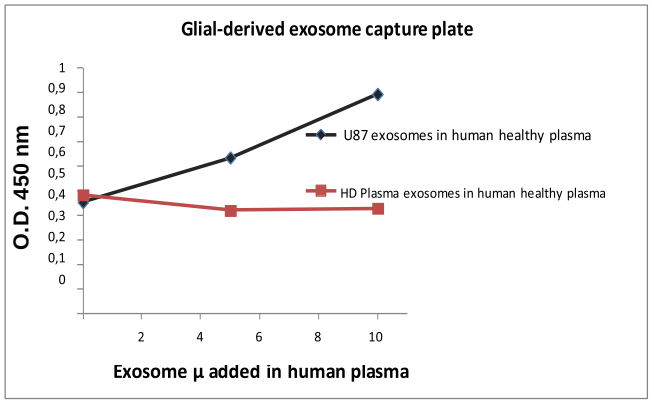

Figure 7. Immunocapture of glioblastoma derived exosomes (U87) subpopulation diluted in human plasma from healthy donors.