Product Information & Manual

Immunoplate for total Exosome Isolation (CD9, Colorimetric)
NBP3-14804

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Novus kits are guaranteed for 6 months from date of receipt.

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PRODUCT DESCRIPTION

Product overview

Immunoplates are 96 multiwell plates covalently pre-coated with specific exosome-binding antibodies allowing exosome capture and isolation from different sources (cell supernatant, human plasma, serum, urine and saliva). Covalent coating improves the correct orientation of antibodies maximizing the quantity of immunocaptured exosomes and increasing the binding efficiency of the plate. ELISA Immunoplates are blocked and stabilized for long term storage.

The ELISA Immunoplates are designed to capture exosomes from biological samples up to 100 µl per well, if necessary adjust the well volume with 1x PBS. Hereby proposed protocol is applicable to all immunoplates presented in the catalog.

Plates are precoated 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.

ELISA immunoplates are ready to use.

About Exosomes

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 amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

Type of Immunoplates available:

- Immunoplate for Overall Exosome capture from human serum (NBP3-14804)
- Immunoplate for Overall Exosome capture from Cell culture media (NBP3-14805)
- Immunoplate for Tumor-derived Exosome capture from human plasma (NBP3-14806)
- Immunoplate for Neural-derived Exosome capture and enrichment (NBP3-14803)
- Immunoplate for Glial-derived Exosome capture and enrichment (NBP3-14802)

Transparent, white and black plates are available depending on the downstream detection approach (colorimetric, luminometric and fluorimetric).

Content	Standard 96-well format pre-coated plate
Expiration date	24 months, store at 4°C 6 month after opening

PROCEDURE FOR SAMPLE PREPARATION AND EXOSOME BINDING

Sample preparation:

Plasma and serum samples preparation

Prepare samples by 3 centrifugation steps to eliminate red blood cells and cellular debris:

- 10' at 300 g
- 20' at 1 200 g
- 30' at 10 000 g

After each step save the supernatant. Processed serum is ready to be loaded onto the plate (100 µl/well). Plasma can be diluted 1/1 in PBS 1x.

Urine samples preparation

Preclear urine samples by centrifugation at 16 000 g for 20' at RT

- Filter by using 0.45 um filter
- Concentrate urine samples by spin concentrator for 15-20 times for proteomic and for nucleic acid studies*.

Cell culture mediaum samples preparation

Prepare cell supernatants by 3 centrifugation steps:

- 10' at 300 g
- 20' at 1 600 g
- 30' at 10 000 g

Concentrate cell supernatant 10-20 times in spin concentrator*

**The quantity of exosomes could vary between samples. Concentration factors are given for information purposes only, a larger starting amount of sample should be used if the signal is weak.*

Saliva samples preparation

Add PBS 1X in saliva samples in ratio 1/1 (5 ml of saliva + 5 ml of PBS 1X). Mix together and proceed to the following centrifugation steps:

- 15' at 2600 g
- 20' at 15550 g
- filter through 0.22 um filter

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.

- The plate is ready to use, no pre-washing is required.
- Add up to 100 µl of sample per well, adjust with 1X PBS up to 100 µl if necessary.
- Seal the plate with parafilm and incubate at room temperature while shaking for 20 minutes.
- Depending on your sample, incubate overnight:
  - Plasma and serum samples at 4°C.
  - Cell culture supernatant, saliva and urine samples at 37°C.
- 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.**

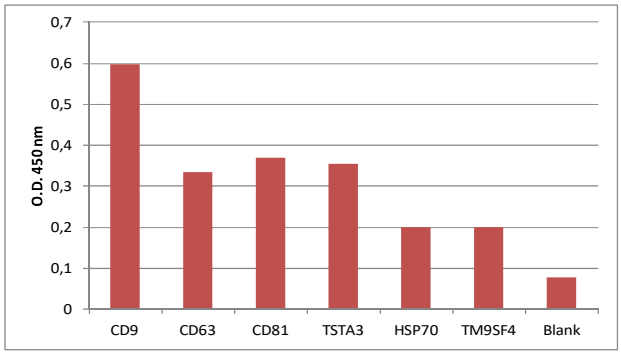
- Wash 3 times with 300 µl/well of Washing Buffer. After each addition, pour off wash. **No Touch.**

- Thus prepared plate can be used for analysis of exosome markers via ELISA assay. Moreover, captured exosomes are suitable for lysisi and extraction of EV-associated RNAs.

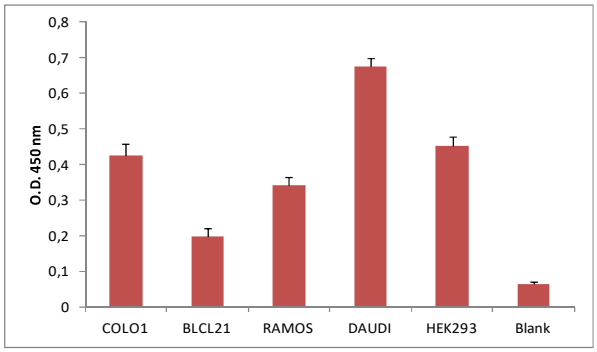
PERFORMANCE

Immunoplates allow exosome protein profiling without pre-purification (by ultracentrifuge, chemical precipitation or microfiltration).

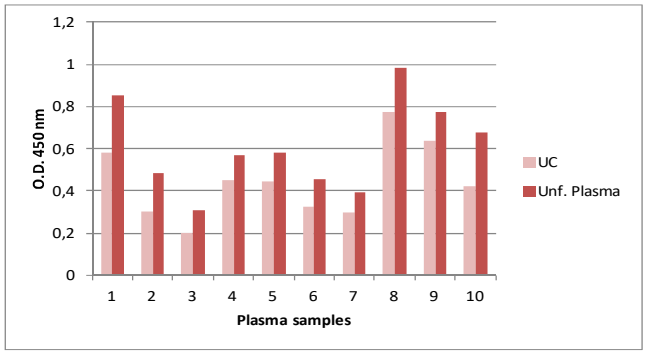
Plates are useful tools for immunocapturing exosomes from biofluids or culture media, for protein analyses and protein marker profiling. They allow quantitative and qualitative simultaneous analysis of different protein markers (Fig 1) from the same sample, or expression profiling of a single marker in different samples (Fig 2), without exosome pre-purification via ultracentrifuge or other methods. CD9 expression (Fig 3) of immunocaptured plasma exosomes mimic that of the correspondent ultracentrifuged fraction. No significant cross-reactivity is observed with soluble antigens or other vesicle-associated proteins (Fig 4).



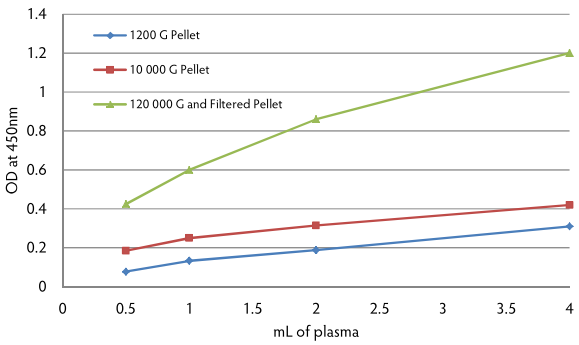
1. Common exosomal biomarkers analysis in a healthy donor's plasma sample



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



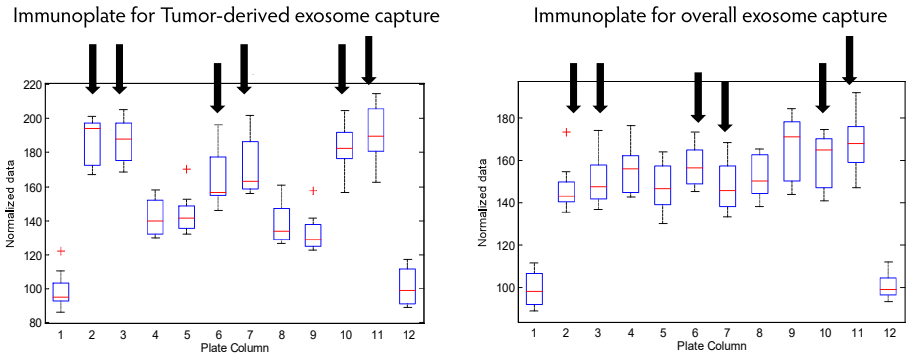
3. Comparison of CD9 detection on purified (ultracentrifuged) plasma exosomes vs unfractionated samples in a set of healthy donor's plasma



4. HBM plate is selective in capturing purified exosomes (pellet after centrifugation 120000g) and no other circulating microvesicle (pellet 1200g and 10000g)

Immunoplates enable enrichment in tumor-derived exosome subpopulation from human plasma

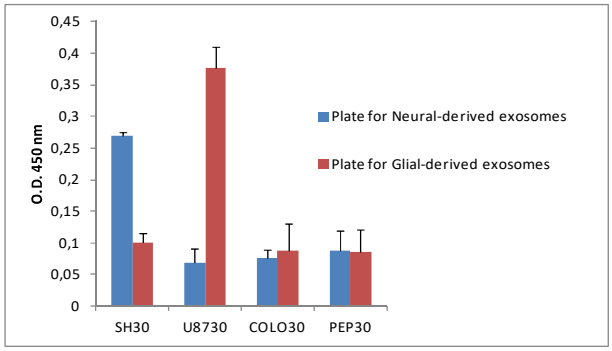
Tumor-derived Exosome capture Immunoplates are precoated with a proprietary capturing antibody which allows enrichment and selection of exosomes from tumor origin from human plasma, particularly useful for cancer biomarker profiling. Figure 5 shows that by simply using the Tumor-derived exosome plate it is possible to discriminate healthy donors from cancer patient (indicated by a black arrows).



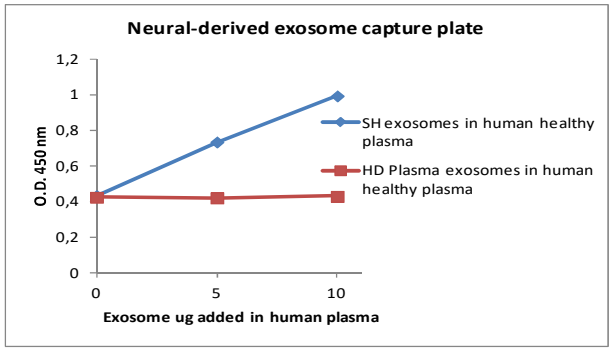
5. Immunoplate for Tumor-derived exosomes capture allows discrimination of cancer patients (black arrows)

Specific Immunoplates for enrichment in neural, glial, platelet-derived exosome subpopulation from human plasma

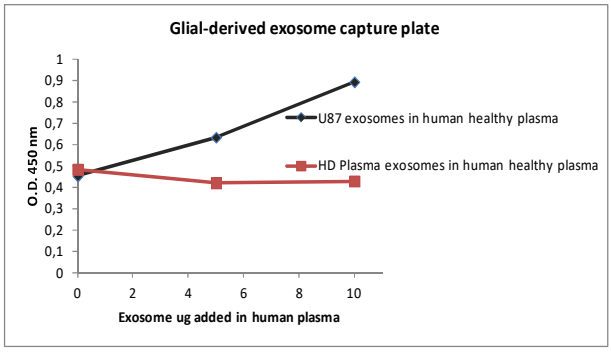
These plates are precoated with exosome associated antibody indicative of neurological, glial or monocyte/platelet origin enabling specific enrichment of exosomes from human plasma samples.



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.



7. Immunocapture of neuroblastoma derived exosomes (SH) subpopulation diluted in human plasma from healthy donors.



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