Immunoprecipitation-based blood extracellular vesicle enrichment protocol

Immunoprecipitation (IP) is a powerful technique used to isolate extracellular vesicles (EVs) from blood based on the presence of specific surface markers. Below is a detailed protocol for the enrichment of blood-derived EVs using immunoprecipitation:

Materials Needed Blood Sample: Typically, plasma or serum. Centrifuge: Capable of high-speed centrifugation. PBS (Phosphate Buffered Saline): Sterile. EV-specific antibodies: Conjugated to beads (e.g., anti-CD63, anti-CD81, or anti-CD9). Magnetic beads or Agarose beads: Conjugated with the specific antibody. Lysis buffer: If required for downstream applications. Wash buffer: PBS or a buffer suitable for the specific antibody used. Elution buffer: For eluting EVs from beads. Proteinase inhibitors: Optional, to prevent proteolysis. Ultracentrifuge tubes: If using ultracentrifugation for initial EV enrichment. Protocol 1. Sample Preparation Blood Collection:

Collect blood in EDTA or citrate tubes to prevent coagulation. Centrifuge the blood at 1,500 x g for 10 minutes at 4°C to separate plasma/serum from cells. Carefully collect the plasma/serum without disturbing the buffy coat. Pre-clear Plasma/Serum:

Centrifuge the plasma/serum at 10,000 x g for 10 minutes at 4°C to remove cell debris and larger particles. 2. Initial EV Isolation (Optional but recommended) Ultracentrifugation (if available): Transfer the pre-cleared plasma/serum to ultracentrifuge tubes. Centrifuge at 100,000 x g for 70 minutes at 4°C. Discard the supernatant and resuspend the EV pellet in PBS. 3. Immunoprecipitation Prepare Antibody-conjugated Beads:

If using magnetic beads, wash the beads three times with PBS or wash buffer. If using agarose beads, wash according to the manufacturer’s instructions. Incubate with Antibodies:

Incubate the resuspended EVs (from step 2) or pre-cleared plasma/serum with antibody-conjugated beads. Rotate the mixture at 4°C overnight to allow binding of EVs to the beads. Washing:

After incubation, separate the beads using a magnetic separator (for magnetic beads) or by centrifugation (for agarose beads). Wash the beads three to five times with PBS or wash buffer to remove unbound materials. Elution of EVs:

Elute the bound EVs from the beads using an elution buffer suitable for downstream applications. Elution conditions depend on the type of beads and antibodies used (e.g., low pH, high salt). 4. Downstream Analysis EV Characterization:

Quantify and qualify the isolated EVs using techniques such as NTA (Nanoparticle Tracking Analysis), TEM (Transmission Electron Microscopy), or western blotting for EV markers (e.g., CD63, CD81, CD9). Perform functional assays if required. Storage:

Store the isolated EVs at -80°C for long-term storage or at 4°C for short-term use. Notes The choice of antibody (anti-CD63, anti-CD81, anti-CD9) depends on the EV surface markers you are targeting. Ensure all reagents and buffers are sterile to avoid contamination. The efficiency of immunoprecipitation can vary based on the antibody and bead quality. It might be necessary to optimize the conditions (e.g., antibody concentration, incubation time) for specific samples. This protocol provides a basic framework for EV enrichment from blood samples using immunoprecipitation. Adjustments and optimizations may be necessary based on specific

experimental needs and sample types.