Migrasome Therapeutics

Migralso™

Total cell-derived migrasome isolation kit for mouse blood

PRMB001 / PRMB001S

Instruction manual

MigraIso™

Total cell-derived migrasome isolation kit for mouse blood

Catalog number	Pack size
PRMB001	20 tests (20 mL mouse plasma)
PRMB001S	5 tests (5 mL mouse plasma)

This product is designed for scientific research and is not intended for clinical diagnosis. Read the detailed risk and liability statement in this manual carefully before using the product.

Product overview

This kit isolates migrasomes from mouse blood or plasma. It can also be used with plasma or serum stored at -80°C. However, the centrifugation speed used to prepare plasma can affect migrasome loss, and freeze-thawing can increase platelet fragments. These factors may affect yield and recovery.

This kit is not suitable for plasma obtained using blood-filtration membranes. The procedure is rapid, efficient, and straightforward, with modest equipment requirements. It produces migrasome preparations of consistent quality with low protein contamination. The isolated samples can be used for migrasome counting, protein extraction and analysis, RNA extraction and analysis, cell function assays, and other biological experiments.

Product contents

Component	PRMB001 (20 tests)	PRMB001S (5 tests)
Buffer A	25 mL	5 mL
Diluent B	80 mL	20 mL
Purification reagent C	160 μL	40 μL
Magnetic bead suspension D	400 μL	100 μL
15 mL centrifuge tubes	20 tubes	5 tubes
Instruction manual	1 copy	1 copy

Reagents and equipment required but not supplied

- Sterile 1× PBS.
- Collection tubes: low-binding or blocked 1.5 mL microcentrifuge tubes and 15 mL centrifuge tubes. The kit includes 15 mL tubes for use at 20,000 × g.
- Low-binding pipette tips.
- Magnetic rack.

Storage and shipping

Transport at 4°C. On receipt, store at 4°C. Do not freeze or allow ice to form. Shelf life: 12 months.

Procedure

A plasma volume of 4 to 5 mL is recommended per extraction. The procedure below uses 10 mL peripheral blood as an example. Low-binding materials, including pipette tips and centrifuge tubes, are recommended throughout the procedure.

1. Enrich the blood migrasome fraction

For plasma or serum samples, prepare the diluent in step 1-1, skip step 1-2, and proceed to step 1-3.

1-1. Prepare the plasma diluent by mixing buffer A and diluent B at 1:9. Prepare twice the volume of the plasma sample to be processed.

1-2. Centrifuge 10 mL peripheral blood at 800 × g for 15 min at room temperature. A swinging-bucket rotor is recommended whenever possible for optimal separation.

Adjust the centrifugation time according to sample volume and the observed separation. Smaller volumes may require less time and larger volumes more time. Distinct plasma (pale yellow), leukocyte (white), and erythrocyte (red) layers should be visible. With a fixed-angle rotor, the pellet forms at an angle and can be disturbed during aspiration, contaminating the supernatant. Handle it carefully.

1-3. Without aspirating the pellet, transfer as much plasma as possible into a new, user-supplied 15 mL centrifuge tube. Centrifuge at 1,000 × g for 5 min at room temperature. A swinging-bucket rotor is recommended whenever possible for optimal separation.

1-4. Without aspirating the pellet, transfer as much plasma as possible into a new 15 mL tube supplied with the kit. Dilute the plasma with the diluent prepared in step 1-1 at a plasma:diluent ratio of 1:2.

1-5. Centrifuge at 20,000 × g for 60 min at 4°C.

The rotor angle is important for separation. A rotor angle of 45° or greater is recommended. At smaller angles, the pellet may adhere to the tube wall instead of collecting at the bottom.

1-6. Remove most of the supernatant, briefly centrifuge, and remove as much residual supernatant as possible while retaining the pellet. This pellet is the enriched blood migrasome fraction. Prepare it according to the downstream assay. Retain the supernatant for blocking in later steps.

2. Purify total cell-derived migrasomes

2-1. Prepare the purification buffer by adding 100 μL buffer A to 900 μL PBS.

2-2. Add the supernatant from step 1-6 to a low-binding microcentrifuge tube. Allow to stand for at least 1 min, then remove the supernatant. Briefly centrifuge and remove as much residual liquid as possible to complete tube blocking.

2-3. Block a 20 μL pipette tip by aspirating and dispensing the supernatant from step 1-6 ten times. Block all tips used to pipette the sample directly in the same way.

2-4. Resuspend the pellet from step 1-6 in 20 μL purification buffer. Resuspend thoroughly, as the extent of resuspension directly affects separation. Transfer the suspension into a blocked, low-binding 1.5 mL microcentrifuge tube.

2-5. Add 8 μL purification reagent C per mL plasma. Mix thoroughly using the 20 μL tip from step 2-4. Incubate at 4°C for 15 min. At 8 min, gently pipette 10 times to mix.

2-6. Add 100 μL purification buffer without mixing. Centrifuge at 20,000 × g for 30 min at 4°C. Remove as much supernatant as possible and retain the pellet for the next step.

The rotor angle is important for separation. A rotor angle of 45° or greater is recommended. At smaller angles, the pellet may adhere to the tube wall instead of collecting at the bottom.

2-7. Resuspend the pellet from step 2-6 in 20 μL purification buffer.

Resuspend the pellet thoroughly. The extent of resuspension directly affects separation.

2-8. Vortex magnetic bead suspension D for at least 10 s to mix thoroughly, then add 20 μL per mL plasma to the sample. Mix thoroughly by pipetting without introducing bubbles. Add 400 μL purification buffer and incubate at 4°C with rotation at approximately 10 rpm for 60 min.

2-9. Using a blocked 1 mL tip, pipette thoroughly 15 times. Place the tube on a magnetic rack at 4°C for 15 min.

2-10. Avoid disturbing the magnetic beads. Using a blocked 200 μL tip, transfer the supernatant into a new, blocked, low-binding 1.5 mL microcentrifuge tube. To ensure complete bead removal, one repeat of the magnetic separation and supernatant transfer in steps 2-9 to 2-10 is recommended before centrifugation. Centrifuge the supernatant at 20,000 × g for 45 min at 4°C.

The rotor angle is important for separation. A rotor angle of 45° or greater is recommended. At smaller angles, the pellet may adhere to the tube wall instead of collecting at the bottom.

2-11. Remove most of the supernatant, briefly centrifuge, then remove as much residual supernatant as possible while retaining the pellet. The pellet contains purified total cell-derived migrasomes. Prepare the sample according to the downstream assay.

Precautions

1. Blood collection: Collect mouse blood into tubes containing EDTA anticoagulant. Sodium-citrate-anticoagulated blood has been tested and gives a lower recovery than EDTA-anticoagulated blood. Other anticoagulants have not been tested. Users should evaluate suitability when using another anticoagulant or sample source.

2. Sample storage: Do not store peripheral blood for more than 4 hours before migrasome isolation. Hemolysis may otherwise severely affect separation.

3. Platelet contamination: Minimize mechanical disturbance when handling peripheral blood. Perform the low-speed centrifugation steps before step 1-4 at room temperature, and keep subsequent procedures at 4°C. If platelet contamination is severe, repeat steps 2-1 to 2-11. The amounts of purification reagent C and magnetic bead suspension D may be reduced as appropriate.

4. Rotor selection:

1. For the low-speed centrifugation in steps 1-2 to 1-3, use a swinging-bucket rotor whenever possible. A fixed-angle rotor produces an inclined, loose pellet that can be disturbed and mix into the supernatant.

2. For the high-speed enrichment of peripheral blood migrasomes at 20,000 × g in step 1-5, use a fixed-angle rotor with an angle of 45° or greater whenever possible for the best results.

Important risk and liability statement

This product is for research use only. It has been designed and manufactured in accordance with the standards and regulatory requirements applicable to research reagents. Read this manual carefully before use and comply with all applicable biosafety, legal, ethical, and privacy requirements, including, but not limited to, the following:

- Compliance: Do not use this product for clinical diagnosis, treatment, or any other medical purpose.
- Biosafety: Take appropriate biosafety measures to prevent infection and strictly follow the procedures for handling and storing biohazardous materials.
- Ethics and privacy: Sample collection must comply with all relevant ethical review requirements, protect personal privacy, and be supported by the appropriate informed consent.
- Cross-contamination: Follow this manual to prevent sample cross-contamination and ensure accurate experimental results.
- Storage and stability: Store the product correctly to maintain its stability and effectiveness. Storage outside the specified conditions may reduce performance.
- Waste disposal: Dispose of used reagents and samples safely in accordance with local regulations.
- Performance limitations: Product performance and limitations are described in this manual. Migrasome Therapeutics accepts no responsibility for use outside these instructions.

Migrasome Therapeutics accepts no legal liability for any direct or indirect consequences of improper use of this product or failure to comply with applicable regulations. Contact customer service if you have questions or require further information.

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