Migrasome Therapeutics

# Migralso™

## Human blood B-cell-derived migrasome extraction kit

**PRHB004 / PRHB004S**

**Instruction manual**

# Migralso™

# Human blood B-cell-derived migrasome extraction kit

| Catalog number | Pack size |
|---|---|
| PRHB004 | 20 tests (total OD280 of 100 in the crude preparation per test) |
| PRHB004S | 5 tests (total OD280 of 100 in the crude preparation per test) |

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

## Product overview

This kit uses negative selection to purify B-cell-derived migrasomes from human peripheral blood or plasma samples. It can also be used with plasma stored at -80°C. Yield and recovery may be affected by differences in migrasome loss during plasma collection at different centrifugation speeds and by increased platelet fragmentation after freezing and thawing.

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

# Kit contents

| Component | PRHB004 (20 tests) | PRHB004S (5 tests) |
|---|---|---|
| Promoting reagent A | 32 μL | 8 μL |
| Buffer B | 20 mL | 5 mL |
| Diluent C | 80 mL | 20 mL |
| Purification reagent D | 120 μL | 30 μL |
| Purification reagent E | 20 μL | 5 μL |
| Purification reagent F | 200 μL | 50 μL |
| Magnetic bead suspension G | 300 μL | 75 μL |
| Magnetic bead suspension H | 200 μL | 50 μL |
| 15 mL centrifuge tubes | 20 tubes | 5 tubes |
| Instruction manual | 1 copy | 1 copy |

## Reagents and equipment required but not supplied

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

## Storage and shipping

Transport at 4°C. Upon receipt, store reagents B to H at 4°C. Do not freeze or allow ice to form. Store reagent A at -20°C. Shelf life: 12 months. Promoting reagent A is a suspension; visible sediment is normal. Mix thoroughly before use.

# Procedure

Processing 1 to 3 tests of plasma per extraction is recommended. Low-binding consumables, including pipette tips and centrifuge tubes, are recommended throughout the procedure.

# 0 Plasma preparation

If plasma must be prepared from peripheral blood, use the following recommended procedure.

1. Centrifuge the peripheral blood sample at 800 × g at room temperature. Select the centrifugation time according to sample volume; 15 min may be used for 10 mL of peripheral blood. Use a swinging-bucket rotor where possible for optimal separation.

Adjust the centrifugation time according to sample volume and the observed separation. Smaller samples may require a shorter spin, and larger samples may require a longer spin. The aim is to obtain clearly separated plasma (pale yellow), leukocyte (white), and erythrocyte (red) layers. A fixed-angle rotor produces a sloping pellet that can be disturbed and carried into the supernatant during aspiration. Aspirate carefully.

2. Transfer as much of the plasma layer as possible to a new 15 mL centrifuge tube (not supplied), without aspirating the pellet. Centrifuge at 2,000 × g at room temperature. Select the centrifugation time according to sample volume; 5 min may be used for a sample originating from 10 mL of peripheral blood. Use a swinging-bucket rotor where possible for optimal separation.

3. Transfer as much of the plasma layer as possible to a new 15 mL tube supplied with the kit, without aspirating the pellet.

Tube preparation: Retain the supernatant from step 1-4. Before step 1-5, add it to a low-binding microcentrifuge tube and let it stand for at least 1 min. Remove the supernatant, briefly centrifuge, and aspirate as much residual liquid as possible.

# 1 Enrichment of the blood migrasome fraction

1. Vortex Promoting reagent A for at least 10 s to mix thoroughly. This reagent is a suspension, and visible sediment is normal. Add it to the plasma at a Promoting reagent A:plasma ratio of 1:1000. Mix by gently inverting the tube 10 times.

2. Prepare plasma diluent by mixing Buffer B and Diluent C at a ratio of 1:9. Prepare a total volume equal to three times the plasma volume plus 1.5 mL. Dilute the plasma at a plasma:diluent ratio of 1:3 and reserve 1.5 mL of the prepared diluent for subsequent resuspension.

3. Centrifuge at 20,000 × g for 60 min at 4°C.

Rotor angle affects 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.

4. Remove most of the supernatant, briefly centrifuge, and aspirate as much of the remaining supernatant as possible. Retain the pellet. This pellet is the enriched blood migrasome fraction and may be prepared according to the requirements of the downstream assay. Retain the supernatant for use as a blocking solution in subsequent steps.

5. Resuspend the pellet in 1 mL of the plasma diluent reserved in step 1-2. Transfer the suspension to a blocked microcentrifuge tube and centrifuge again at 20,000 × g for 60 min at 4°C. Remove most of the supernatant, briefly centrifuge, and aspirate as much of the remaining supernatant as possible. Retain the pellet.

# 2 Purification of B-cell-derived migrasomes

1. Vortex Promoting reagent A for at least 10 s to mix thoroughly. This reagent is a suspension, and visible sediment is normal. Prepare purification buffer by mixing Promoting reagent A, Buffer B, and PBS at a ratio of 1:100:900. Approximately 600 μL of purification buffer is required per extraction.

2. Use a low-binding microcentrifuge tube blocked as described under Tube preparation.

3. Block a 20 μL pipette tip by aspirating and dispensing the supernatant from step 1-4 ten times. Block all tips that will directly pipette the sample in the same way.

4. Resuspend the pellet from step 1-5 in 20 μL of purification buffer. Pipette thoroughly until no visible particles remain; the extent of resuspension directly affects separation. Transfer the suspension to a blocked, low-binding 1.5 mL centrifuge tube.

5. Use 1 μL of the crude preparation to measure A280 (OD280) on a NanoDrop. Two or three replicate measurements are recommended.

6. Use the measured A280 to calculate the amounts of Purification reagents D, E, and F and Magnetic bead suspensions G and H to add. A sample with total OD280 = 100 is recommended. Total OD280 is calculated as A280 × sample volume in μL; this amount is defined as one test for this kit.

7. Add 6 μL of Purification reagent D, 1 μL of Purification reagent E, and 10 μL of Purification reagent F per test. Mix thoroughly using the 20 μL pipette tip used in step 2-4. Incubate at 4°C for 15 min. At 8 min, mix once by gently pipetting 10 times.

8. Add 100 μL of purification buffer. Mixing is not required. Centrifuge at 20,000 × g for 20 min at 4°C. Aspirate as much supernatant as possible and retain the pellet for the next step.

Rotor angle affects 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.

9. Resuspend the pellet from step 2-8 in 20 μL of purification buffer.

Pipette thoroughly until no visible particles remain. The extent of resuspension directly affects separation.

10. Vortex Magnetic bead suspensions G and H for at least 10 s to mix thoroughly. Add 15 μL of Magnetic bead suspension G and 10 μL of Magnetic bead suspension H per test to the sample. Mix thoroughly by pipetting without introducing bubbles. Add 400 μL of purification buffer and incubate at 4°C for 60 min with rotation at 5 to 10 rpm.

11. Pipette thoroughly 15 times using a blocked 1 mL pipette tip. Place the tube on a magnetic rack at 4°C for 15 min.

12. Without disturbing the magnetic beads, use a blocked 200 μL pipette tip to transfer the supernatant to a new, blocked, low-binding 1.5 mL centrifuge tube. To ensure thorough bead removal, repeating the magnetic separation and supernatant transfer in steps 2-11 and 2-12 once is recommended before centrifugation. Centrifuge the supernatant at 20,000 × g for 45 min at 4°C.

Rotor angle affects 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.

13. Remove most of the supernatant, briefly centrifuge, and aspirate as much of the remaining supernatant as possible. Retain the pellet. This pellet contains the purified B-cell-derived migrasomes and may be prepared according to the requirements of the downstream assay.

# Precautions

1. Blood collection: Collect peripheral blood in sodium citrate anticoagulant tubes. This kit has been tested with EDTA-anticoagulated blood, which showed more platelet contamination than sodium citrate-anticoagulated blood. Blood collected with other anticoagulants has not been tested. Evaluating suitability before using other anticoagulants or sample sources is recommended.

2. Sample storage: Fresh plasma and plasma stored at -80°C can be used with this kit. Peripheral blood intended for plasma preparation must not be stored for more than 4 h before migrasome extraction; hemolysis may otherwise severely affect separation.

3. Platelet contamination: Minimize mechanical disturbance when handling peripheral blood. Perform the low-speed centrifugation in section 0, Plasma preparation, at room temperature. Keep operations after step 1-4 at 4°C. If platelet contamination is severe, steps 2-4 to 2-13 may be repeated. The amounts of Purification reagent D and Magnetic bead suspension G may be reduced as appropriate.

4. Centrifuge rotor selection:

a. Use a swinging-bucket rotor where possible for low-speed centrifugation in steps 0-1 and 0-2. A fixed-angle rotor produces a sloping, loosely packed pellet that is easily disturbed and carried into the supernatant.

b. For crude 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 where possible for optimal results.

# Important risk and liability statement

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

1. Compliance: This product must not be used for clinical diagnosis, treatment, or any other medical purpose.
2. Biosafety: Take appropriate biosafety measures to avoid infection risks and strictly follow procedures for handling and storing biohazardous materials.
3. Ethics and privacy: Sample collection must comply with all relevant ethical review requirements. Protect personal privacy and obtain the required informed consent.
4. Cross-contamination: Follow this manual to avoid sample cross-contamination and ensure the accuracy of experimental results.
5. Storage and stability: Store the kit correctly to maintain its stability and effectiveness. Storage outside the specified conditions may reduce performance.
6. Waste disposal: Dispose of used reagents and samples safely in accordance with local regulations.
7. Product performance limitations: This manual describes product performance and limitations in detail. Migrasome Therapeutics accepts no responsibility for use outside the instructions in this manual.

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 need further information.

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