

Migralso™

Human blood neutrophil-derived migrasome extraction kit

PRHB002 / PRHB002S

Instruction manual

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Catalog number	Pack size
PRHB002	20 tests (total OD280 of 100 in the crude preparation per test)
PRHB002S	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 purifies 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	PRHB002 (20 tests)	PRHB002S (5 tests)
Optimization reagent A	20 μ L	5 μ L
Enhancing reagent B	112 μ L	28 μ L
Promoting reagent C	32 μ L	8 μ L
Buffer D	25 mL	5 mL
Diluent E	80 mL	20 mL
Purification reagent F	120 μ L	30 μ L
Purification reagent G	20 μ L	5 μ L
Purification reagent H	80 μ L	20 μ L
Magnetic bead suspension I	600 μ L	150 μ L
Magnetic bead suspension J	100 μ L	25 μ L
15 mL centrifuge tubes	20 tubes	5 tubes
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Workflow (1/3)

Green: retain. Dashed amber: save supernatant for blocking.

0-1



Plasma



Separate plasma by centrifugation.

800 × g, room temperature

Adjust the time to sample volume and separation; see note 1.

Retain the plasma layer

0-2



Plasma



Transfer the plasma and centrifuge again.

New 15 mL tube (user supplied)

2,000 × g, room temperature

Avoid the pellet; see note 1 for centrifugation time.

Retain the plasma layer

0-3; 1-1–1-2



Sample



Transfer the plasma; add A and B and incubate. Add C and mix.

New kit-supplied 15 mL tube

A:B:plasma = 1:4:1000

37°C, 10 min + 10 min

C:plasma = 1:1000

Avoid the pellet during transfer; mix between incubations, as described in note 2.

Retain the mixture

1-3



Sample



Prepare diluent and dilute the plasma.

D:E = 1:9

Plasma:prepared diluent = 1:3

Base the total volume on the starting sample; reserve the remainder for step 1-7.

See note 3.

Retain the diluted sample

1-4–1-5



Pellet

Save supernatant
for blocking

Centrifuge and retain the pellet.

20,000 × g, 60 min, 4°C

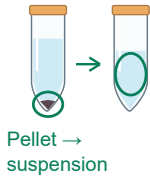
Reserve supernatant for blocking in steps 1-6 and 2-2.

Keep the pellet; reserve the supernatant separately for blocking

Workflow (2/3)

Green: retain. Dashed amber: save supernatant for blocking.

1-6–1-7; 2-1–2-3



Resuspend; transfer to a blocked tube and centrifuge. Resuspend the pellet.

1 mL reserved diluent (step 1-3)

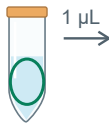
20,000 × g, 60 min, 4°C

Resuspend in 50 µL purification buffer

See notes 4 and 5 for blocking and purification buffer preparation. Resuspend thoroughly until no visible particles remain.

Retain the pellet, then the resuspended sample

2-4



Measure A280 of the crude preparation.

1 µL sample; NanoDrop

Two or three replicate measurements are recommended.

Retain the remaining sample

2-5–2-6



Calculate reagent amounts from total A280; add B and mix.

Total A280 of 100 = 1 test

Add 1.6 µL B per test

A total A280 of 100–300 per run is recommended; see note 6. Use a pipette tip blocked in step 2-2.

Retain the mixture

2-7



Add F, G and H; mix and incubate.

Per test: 6 µL F + 1 µL G + 4 µL H

4°C, 15 min

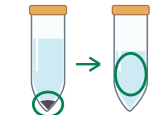
At 8 min, gently pipette 10 times using the tip blocked in step 2-2.

Retain the mixture

Workflow (3/3)

Green: retain. Dashed amber: save supernatant for blocking.

2-8–2-9



Pellet →
suspension

Add purification buffer, centrifuge and resuspend the pellet.

Add 500 μ L purification buffer

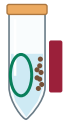
20,000 $\times$ g, 20 min, 4°C

Resuspend in 20 μ L purification buffer

Mixing is not required after adding the 500 μ L. Resuspend thoroughly until no visible particles remain.

Retain the pellet, then the resuspended sample

2-10–2-11



Supernatant

Avoid beads

Add I and J, incubate, then separate on a magnetic rack.

Per test: 30 μ L I + 5 μ L J

Add 400 μ L purification buffer

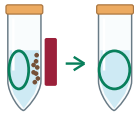
4°C, 5–10 rpm, 60 min

Magnetic separation: 4°C, 15 min

See note 7 for vortexing and pipetting instructions.

Retain the supernatant; leave the beads at the tube wall

2-12 (supernatant transfer)



Supernatant → new
tube

Transfer the supernatant to a new, blocked, low-binding tube.

1.5 mL tube; blocked 200 μ L tip

Avoid disturbing the beads. Repeating magnetic separation and supernatant transfer once before centrifugation is recommended.

Retain the supernatant

2-12 (centrifugation)–2-13



Pellet

Centrifuge, remove the supernatant and retain the pellet.

20,000 $\times$ g, 45 min, 4°C

See note 8 for supernatant removal.

Retain the purified neutrophil-derived migrasome pellet

Workflow notes and reagent key

1. Steps 0-1–0-3 are recommended when preparing plasma from peripheral blood. Adjust centrifugation times to sample volume and separation. For a sample starting with 10 mL peripheral blood, 0-1 may use 15 min and 0-2 may use 5 min.
 2. After adding A and B, gently invert 10 times and incubate at 37°C for 10 min. Gently invert another 10 times and incubate for a further 10 min. Vortex C for at least 10 s before adding it, then gently invert the sample 10 times.
 3. Calculate the total diluent volume from the starting sample: $2 \times \text{peripheral blood volume} + 1.5 \text{ mL}$, or $3 \times \text{plasma volume} + 1.5 \text{ mL}$. Prepare it at D:E = 1:9. Dilute at plasma:prepared diluent = 1:3 and reserve the remainder for resuspension in step 1-7.
 4. Use the supernatant reserved in step 1-5 for blocking. Leave it in a low-binding tube for at least 1 min, remove it, briefly centrifuge and aspirate as much residual liquid as possible. With a 100 μL pipette tip, aspirate and dispense this supernatant 10 times. Block all subsequent tips that directly contact the sample in the same way.
 5. Purification buffer: vortex C for at least 10 s, then prepare C:D:PBS = 1:100:900. Approximately 1000 μL is required per extraction. Purification buffer is distinct from purification reagents F/G/H.
 6. Total A280 = measured A280 $\times$ sample volume in μL . A total A280 of 100 defines one test. A total A280 of 100–300 per run is recommended.
 7. Vortex I and J separately for at least 10 s before addition. Gently pipette the sample thoroughly without introducing bubbles, then add 400 μL purification buffer and incubate with rotation. Before magnetic separation, pipette 15 times with a blocked 1 mL tip.
 8. In steps 1-5, 1-7 and 2-13, remove most of the supernatant, briefly centrifuge and aspirate as much residual liquid as possible; retain the pellet. Reserve supernatant from 1-5 separately for blocking. In step 2-8, aspirate as much supernatant as possible and retain the pellet.
 9. A swinging-bucket rotor is recommended where possible for low-speed centrifugation in steps 0-1/0-2. A rotor angle of at least 45° is recommended for high-speed centrifugation in steps 1-4, 2-8 and 2-12.
- A: Optimization reagent; B: Enhancing reagent; C: Promoting reagent; D: Buffer; E: Diluent; F/G/H: Purification reagents; I/J: Magnetic bead suspensions.

Workflow overview. Follow the corresponding detailed steps and precautions.

Schematic only; liquid volumes are not to scale.

Reagents, equipment and storage

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 D to J at 4°C. Do not freeze or allow ice to form. Store reagents A to C at -20°C. Shelf life: 12 months. Promoting reagent C 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 $800 \times g$ 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 $2,000 \times g$ 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.

1 Enrichment of the blood migrasome fraction

1. Add Optimization reagent A and Enhancing reagent B at an A:B:plasma ratio of 1:4:1000. Mix by gently inverting the tube 10 times and incubate at 37°C for 10 min. Gently invert the tube another 10 times and incubate at 37°C for a further 10 min.

Enhancing reagent B is a pale yellow suspension. After thawing, or if visible sediment is present, vortex thoroughly before use. This does not affect reagent performance.

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

3. Prepare plasma diluent by mixing Buffer D and Diluent E at a ratio of 1:9. Calculate the total volume from the starting sample: twice the peripheral blood volume plus 1.5 mL, or three times the plasma volume plus 1.5 mL. Dilute the plasma at a plasma:diluent ratio of 1:3 and reserve the remaining diluent for subsequent resuspension.

4. Centrifuge at $20,000 \times g$ 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.

5. 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.

6. Add the supernatant from step 1-5 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 supernatant as possible to block the tube.



7. Resuspend the pellet in 1 mL of the plasma diluent reserved in step 1-3. Transfer the suspension to a blocked microcentrifuge tube and centrifuge again at $20,000 \times g$ 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 neutrophil-derived migrasomes

1. Vortex Promoting reagent C 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 C, Buffer D, and PBS at a ratio of 1:100:900. Approximately 1000 μL of purification buffer is required per extraction.
 2. Block a 100 μL pipette tip by aspirating and dispensing the supernatant from step 1-5 ten times. Block all tips that will directly pipette the sample in the same way.
 3. Add 50 μL of purification buffer to the pellet from step 1-7. Pipette thoroughly to resuspend the pellet until no visible particles remain; the extent of resuspension directly affects separation.
 4. Use 1 μL of the crude preparation to measure A280 (OD280) on a NanoDrop. Two or three replicate measurements are recommended.
 5. Use the measured A280 to calculate the amounts of purification reagents and magnetic bead suspensions to add. Processing a sample with a total A280 of 100 to 300 at one time is recommended. Total A280 is calculated as $\text{A280} \times \text{sample volume in } \mu\text{L}$; this kit defines a total A280 of 100 as one test.
- Using a sample with an excessively high total A280 for negative selection may increase platelet-fragment contamination in the final preparation.
6. Add 1.6 μL of Enhancing reagent B per test to the crude preparation. Mix thoroughly using the tip blocked in step 2-2.

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

8. Add 500 μL of purification buffer. Mixing is not required. Centrifuge at 20,000 $\times$ 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 I and J for at least 10 s to mix thoroughly. Add 30 μL of Magnetic bead suspension I and 5 μL of Magnetic bead suspension J per test to the sample. Mix gently and 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 μ L 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 $20,000 \times g$ 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 neutrophil-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 1-5 at 4°C . If platelet contamination is severe, steps 2-1 to 2-13 may be repeated. The amounts of Purification reagent F and Magnetic bead suspension I 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 $20,000 \times g$ in step 1-4, 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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