

# Migralso™ Tissue

Tissue migrasome purification kit

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**PRT001 / PRT001S**

**Instruction manual**

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Tissue migrasome purification kit

| Catalog number | Pack size                               |
|----------------|-----------------------------------------|
| PRT001         | 50 tests (50 × 100 µg crude migrasomes) |
| PRT001S        | 10 tests (10 × 100 µg crude migrasomes) |

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 uses a new method that combines antibodies against intracellular membranes with magnetic beads to remove intracellular membrane contaminants from crude migrasome preparations. It reduces contamination by cell debris and intracellular membranes generated during tissue dissociation, producing migrasomes of higher purity.

## Product contents

| Component                  | PRT001 (50 tests) | PRT001S (10 tests) |
|----------------------------|-------------------|--------------------|
| Purification reagent A     | 250 $\mu$ L       | 50 $\mu$ L         |
| Magnetic bead suspension B | 5 mL              | 1 mL               |
| Instruction manual         | 1 copy            | 1 copy             |

## Materials required but not supplied

- Reagents for tissue dissociation and preparation of a single-cell suspension.
- Sterile 1 $\times$  PBS.
- Collection tubes: low-binding 1.5 mL microcentrifuge tubes, 15 mL centrifuge tubes, and 50 mL centrifuge tubes. Before using the microcentrifuge tubes, add 2% BSA, allow to stand for at least 1 min, then remove the supernatant.
- Low-binding pipette tips.
- Magnetic rack.

## Storage and shipping

Transport and store at 4°C. Shelf life: 6 months. Purification reagent A can be stored at -20°C for a shelf life of up to 12 months; avoid repeated freeze-thaw cycles. Do not allow magnetic bead suspension B to freeze.

## Procedure

### 1. Prepare a single-cell suspension

1-1. Dissociate the tissue using an enzymatic digestion buffer and method appropriate to the tissue type.

1-2. Pass the preparation through a 70  $\mu\text{m}$  strainer to obtain a single-cell suspension. A volume of 10 mL in a 15 mL tube is recommended.

### 2. Isolate crude migrasomes

2-3. Centrifuge at  $1,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ . Collect the supernatant.

2-4. Centrifuge at  $4,000 \times g$  for 5 min at  $4^{\circ}\text{C}$ . Collect the supernatant.

Alternative low-speed centrifugation conditions may be tested, or filtration may be used in place of this centrifugation step.

2-5. Centrifuge at  $18,000 \times g$  for 60 min at  $4^{\circ}\text{C}$ . Remove the supernatant.

### 3. Purify migrasomes

3-6. Resuspend the pellet from step 2-5 in approximately 50 to 100  $\mu\text{L}$  PBS, depending on the amount of pellet. Measure the protein concentration using a BCA assay, then transfer an appropriate amount of the crude migrasome preparation into a new microcentrifuge tube.

3-7. Add 5  $\mu\text{L}$  purification reagent A per 100  $\mu\text{g}$  crude migrasomes. Using a low-binding pipette tip, gently and thoroughly pipette 10 times until no visible particles remain. The extent of resuspension directly affects separation.

The amount of purification reagent A required can vary substantially between tissues. The table below lists amounts tested by Migrasome Therapeutics for reference.

| Tissue                | Reagent A per 100 µg crude migrasomes |
|-----------------------|---------------------------------------|
| Mouse lymph node      | 2 to 4 µL                             |
| Mouse spleen          | 2 to 4 µL                             |
| Mouse 4T1 solid tumor | 5 to 10 µL                            |

3-8. Incubate at 4°C for 15 min. At 8 min, gently pipette 10 times using a low-binding tip.

3-9. Centrifuge at 18,000 × g for 20 min at 4°C. Remove as much supernatant as possible and retain the pellet.

3-10. Add 100 µL PBS and resuspend the pellet thoroughly by pipetting until no visible particles remain. The extent of resuspension directly affects separation.

3-11. Vortex magnetic bead suspension B for at least 10 s to mix thoroughly. Add 20 µL magnetic bead suspension B per 1 µL purification reagent A used. If the total volume is below 500 µL, add PBS to bring it to 500 µL.

3-12. Incubate with rotation at 10 to 15 rpm for 45 min at 4°C.

3-13. Using a low-binding 1 mL tip, pipette gently and thoroughly 5 times. Place the tube on a magnetic rack at 4°C for 15 min.

3-14. Avoid disturbing the magnetic beads. Using a low-binding 200 µL tip, transfer the supernatant into a new, low-binding 1.5 mL microcentrifuge tube blocked as described under Materials required but not supplied. To ensure complete bead removal, one repeat of the magnetic separation and supernatant transfer in steps 3-13 to 3-14 is recommended before centrifugation. Centrifuge the supernatant at 18,000 × g for 45 min at 4°C.

3-15. Remove most of the supernatant, briefly centrifuge, then remove as much residual supernatant as possible while retaining the pellet. The pellet contains purified migrasomes. See precaution 4 for downstream experiments and storage.

## Precautions

1. Tissue-dissociation reagents are not supplied. Users must dissociate the tissue and prepare a single-cell suspension. Migrasome recovery and contamination vary with the dissociation method; evaluate the method experimentally.
2. A swinging-bucket rotor is recommended for low-speed centrifugation. A fixed-angle rotor with an angle greater than 45° is recommended for high-speed centrifugation.
3. The recommended input per test is 100 µg crude migrasomes. Larger or smaller inputs may be tested by scaling the reagent amounts according to the ratios in the procedure, although this may affect recovery. Recovery is generally approximately 5% to 25% and can vary substantially between tissues.

| Tissue                | Recovery   |
|-----------------------|------------|
| Mouse lymph node      | 5% to 15%  |
| Mouse spleen          | 5% to 15%  |
| Mouse 4T1 solid tumor | 15% to 25% |

4. Select storage conditions for crude and purified migrasomes according to the downstream experiment. For analyses of migrasome contents, such as nucleic acid extraction, western blotting, or mass spectrometry, remove the supernatant after high-speed centrifugation and store the pellet at -80°C. For experiments involving morphology, number, surface properties, or activity, such as confocal imaging, vesicle counting, flow cytometry, conventional negative-stain electron microscopy, or cell function assays, storage at 4°C is recommended. Repeated testing has shown that storage at 4°C for up to one week has little effect on migrasome morphology and number. Freezing at -20°C or -80°C causes aggregation and some rupture; the effects vary with the cell source. If freezing is necessary, a preliminary experiment to evaluate its effects is recommended.

## 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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