

MigraPure™ Turbo

Density-gradient-free rapid
cultured-cell migrasome purification kit

PRC005 / PRC005S

Instruction manual

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Catalog number	Pack size
PRC005	10 tests (10 × 50 µg crude migrasomes)
PRC005S	2 tests (2 × 50 µg crude migrasomes)

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

Product overview

This kit is intended for isolating migrasomes from cultured cells. It uses an immunomagnetic negative-selection system developed and optimized by Migrasome Therapeutics to remove endomembrane-derived contaminants from crude migrasomes and achieve purification comparable to conventional density-gradient centrifugation.

The procedure is rapid, efficient and straightforward. It yields migrasomes of consistent quality with low protein contamination for biological experiments, including migrasome counting, protein extraction and analysis, RNA extraction and analysis, and cell function assays.

Kit contents

Component	PRC005 (10 tests)	PRC005S (2 tests)
Purification reagent A	250 μ L	50 μ L
Magnetic bead suspension B	500 μ L	100 μ L
Instruction manual	1 copy	1 copy

Reagents and equipment supplied by the user

1. Cell culture reagents and consumables, including trypsin and culture dishes.
2. Sterile 1 $\times$ PBS.
3. Collection tubes: low-binding 1.5 mL microcentrifuge tubes, 15 mL centrifuge tubes and 50 mL centrifuge tubes.
4. Low-binding pipette tips.
5. Magnetic separation rack.

Storage and shipping

Ship and store at 4°C. Shelf life is 6 months. Purification reagent A may be stored at -20°C to extend its shelf life to 12 months; avoid repeated freezing and thawing. Magnetic bead suspension B must not be allowed to freeze.

Procedure

1. Resuspend the crude migrasome pellet in an appropriate volume of PBS for the pellet size. Measure the protein concentration using a BCA assay and transfer an appropriate amount of crude migrasomes to a fresh microcentrifuge tube.

Reference yield: approximately 5-10 µg crude migrasomes per 15 cm dish of L929 Tspan4-GFP cells.

2. Add 5 µL purification reagent A per 10 µg crude migrasomes. Mix gently and thoroughly by pipetting 10 times with a low-binding tip.

Pipette thoroughly until no visible particles remain. The completeness of mixing directly affects separation performance.

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

4. Centrifuge at $18,000 \times g$ for 20 min at 4°C. Remove as much supernatant as possible and retain the pellet for the next step.

5. Add 100 µL PBS and resuspend the pellet thoroughly.

Resuspend the pellet thoroughly by pipetting until no visible particles remain. The completeness of resuspension directly affects separation performance.

Procedure (continued)

6. Vortex magnetic bead suspension B for at least 10 s to mix thoroughly. Add 10 μL suspension B per 10 μg crude migrasomes and adjust the total volume to 500 μL with PBS.
7. Incubate at 4°C for 45 min with rotation at 10-15 rpm.
8. Pipette gently and thoroughly 5 times using a low-binding 1 mL tip. Place the tube on a magnetic rack at 4°C for 15 min. Magnetic separation may be repeated once to improve removal of the beads.
9. Transfer the supernatant to a fresh low-binding 1.5 mL centrifuge tube using a low-binding 200 μL tip, disturbing the beads as little as possible. Centrifuge the supernatant at 18,000 $\times g$ for 45 min at 4°C.
10. Remove most of the supernatant, briefly centrifuge the tube, then remove as much remaining supernatant as possible. Retain the pellet, which contains purified cultured-cell migrasomes. See Precaution 7 for downstream applications and storage.

Precautions

1. Migratory cell lines and primary cells can produce migrasomes. Production capacity depends on the cells and must be assessed by the user. Adherent cells with strong migratory capacity are recommended, including cells that become adherent and more migratory after overexpression of Tspan4 or other genes that promote migrasome formation. Suspension cells are not recommended, although extracellular matrix coating or gene editing may be tested to promote adhesion. Before routine migrasome production, in situ WGA staining and microscopic examination of migrasome formation are recommended.
2. Migrasomes form during cell migration, so cultures must not be too dense. Based on our experience, aim for 20%-40% confluence at harvest, taking cell migration speed into account. Use a lower density for faster-moving cells.
3. Control the production time closely. Both excessively short and excessively long production times reduce production efficiency. Based on our experience, approximately 12-16 h is recommended.
4. Migrasome production depends on interactions between the cell surface and the culture substrate. Yield may vary with the extracellular matrix and cell type. Optimize the detachment method: insufficient detachment leaves migrasomes behind and reduces yield, whereas excessive digestion or pipetting increases cell disruption and reduces sample purity.
5. A swinging-bucket rotor is recommended for low-speed centrifugation, and a fixed-angle rotor with an angle greater than 45° is recommended for high-speed centrifugation.
6. Under typical conditions, an input of at least 50 µg is recommended. Recovery after purification is approximately 30%-50% for L929 cells and may vary substantially among cell types.

Precautions (continued)

7. Choose 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 migrasome pellet at -80°C . For analyses of migrasome 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 tests showed little effect on migrasome morphology or number during one week at 4°C . Freezing at -20°C or -80°C causes aggregation and some rupture, with differences among cell sources. If freezing is necessary, perform preliminary experiments to assess its effects.

Important risk and liability statement

This kit is for research use only and has been designed and manufactured in accordance with applicable standards and regulatory requirements for research reagents. Read the product manual carefully before use. Users must 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 precautions to prevent infection and strictly follow procedures for handling and storing biohazardous materials.
3. Ethics and privacy: Sample collection must comply with all relevant ethical review standards, protect personal privacy and be covered by appropriate informed consent.
4. Cross-contamination: Follow this manual to prevent sample cross-contamination and ensure accurate experimental results.
5. Storage and stability: Store the kit correctly to maintain its stability and effectiveness. Failure to follow the storage instructions may impair performance.
6. Waste disposal: Dispose of used reagents and samples safely in accordance with local regulations.
7. Product 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 with any questions or requests for further information.

Contact information

Beijing Migrasome Therapeutics Co., Ltd.

Address: 4th Floor, Building 2, No. 9 Yiliaoyuan Road, Changping District, Beijing, China

Postal code: 102200

Telephone: 010-53671078

Email: service@migrasome.com

Website: www.migrasome.com