

MigraPrep™

Cultured-cell migrasome production
and purification kit (density gradient)

PRC001 / PRC001S

Instruction manual

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Catalog number	Pack size
PRC001	50 tests (50 × 150 mm culture dishes)
PRC001S	10 tests (10 × 150 mm culture dishes)

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. 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	PRC001 (50 tests)	PRC001S (10 tests)
Purification reagents A-I	5 mL	1 mL
Matrix solution J	1.5 mL	300 µL
Diluent K	50 mL	10 mL
Resuspension solution L	100 mL	20 mL
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× PBS.
3. Collection tubes: low-binding 1.5 mL microcentrifuge tubes, 15 mL centrifuge tubes and 50 mL centrifuge tubes.
4. A low-speed centrifuge with a swinging-bucket rotor and a high-speed centrifuge with a fixed-angle rotor.
5. 5 mL tubes for density-gradient ultracentrifugation and an ultracentrifuge; see Precaution 8.
6. Low-binding pipette tips.

Storage and shipping

Ship at 4°C. On receipt, store matrix solution J at -20°C and all other reagents at 4°C. Shelf life is 12 months.

Procedure

1. Seed cells

1. Prepare diluted reagent K by mixing PBS and reagent K at a ratio of 9:1.
2. Prepare the coating solution by mixing matrix solution J and diluted reagent K at a ratio of 1:300. Optimize the concentration for the cell system. Add 9 mL per 15 cm dish and incubate at 37°C for at least 1 h.
3. Aspirate the coating solution. Prepare the required number of cells in advance and seed them in the 15 cm dishes at the required density.

Determine the optimal density for migrasome isolation in advance. This density should be substantially lower than the optimal density for routine cell culture. See Precaution 2.

4. Incubate overnight under the appropriate cell culture conditions.

Determine the optimal cell culture conditions in advance. See Precaution 3.

2. Prepare crude migrasomes

1. Discard the culture medium and rinse once with PBS to remove residual medium.
2. Detach cells with an appropriate concentration of trypsin. Add culture medium to stop digestion and collect the cell and migrasome suspension.

Optimize digestion in advance to detach as many intact migrasomes as possible. Pre-blocking the 50 mL collection tube with complete culture medium is recommended to reduce loss through adsorption to the tube wall.

2. Prepare crude migrasomes (continued)

3. Centrifuge at $300 \times g$ for 5 min at 4°C and retain the supernatant.
4. Centrifuge at $2,500 \times g$ for 10 min at 4°C and retain the supernatant.

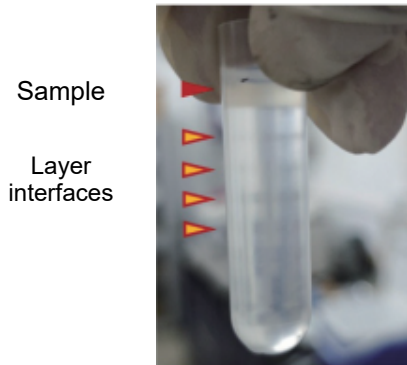
The centrifugation conditions in steps 2.3-2.4 were optimized using L929 cells. Users may test more suitable low-speed centrifugation conditions or replace these centrifugation steps with filtration.

5. Centrifuge at $18,000 \times g$ for 60 min at 4°C and discard the supernatant.

3. Purify migrasomes

1. Resuspend the pellet from step 2.5 in 500 μL gradient solution I. The recommended input is approximately 100 μg ; adjust it according to the purity of the crude preparation. See Precaution 6.
2. Add 500 μL of each gradient solution, in order from A to H, to a 5 mL density-gradient ultracentrifuge tube. Finally, layer the crude migrasomes resuspended in gradient solution I on top. Handle the tube very gently throughout to avoid disturbing the gradient interfaces.

A 1 mL pipette tip with its end cut off using clean scissors may be used to prepare the gradient. To preserve the shelf life of the gradient solutions and prevent subsequent sample contamination, prepare the gradient in a clean bench or biological safety cabinet whenever possible. A correctly prepared gradient has clearly visible interfaces between layers, as shown.



Prepared density gradient with the sample at the top and visible layer interfaces.

Figure labels: Sample; Layer interfaces.

3. Purify migrasomes (continued)

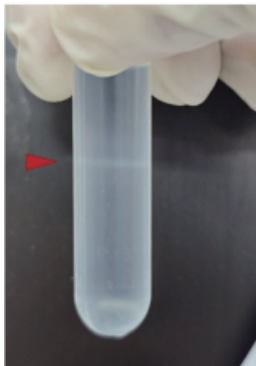
3. Centrifuge at $150,000 \times g$ for 4 h at 4°C .

A Beckman benchtop ultracentrifuge with an MLS-50 rotor is recommended. If this equipment is unavailable, see Precaution 8.

4. After centrifugation, collect layers 3-5 in 500 μL fractions; see the note below. Dilute the sample with reagent L at a sample:reagent L ratio of 1:2. Mix thoroughly and centrifuge at $18,000 \times g$ for 60 min at 4°C .

With a sufficiently large sample, the target band is clearly visible. The enriched layers may differ among cell types. When isolating migrasomes from a cell line for the first time, retain a sample from each layer and analyze the fractions to identify the relevant layers. The L929 cell example below is provided for reference; target bands may differ for other cell samples.

Target
band



Example of the target band in an L929-derived sample after density-gradient centrifugation.

Figure label: Target band.

5. The pellet contains purified migrasomes. See Precaution 9 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 approximately 100 µg is recommended. Each density gradient has a limited loading capacity. Overloading may cause incomplete separation and reduce the purity of the purified preparation. Recovery is approximately 20%-30%. The allowable input also varies substantially with the purity of the crude preparation. Adjust the input if the sample contains large amounts of membrane fragments, intracellular organelles or other impurities.

Precautions (continued)

7. Prepare the density gradient very gently to avoid disturbing the interfaces. Cutting the end off a 1 mL pipette tip with scissors may make gradient preparation easier.
8. If a Beckman benchtop ultracentrifuge with an MLS-50 rotor is unavailable, another ultracentrifuge with a swinging-bucket rotor capable of $150,000 \times g$ may be used. Scale all gradient volumes proportionally to the centrifuge tube. For example, use 1 mL per layer in a 9.5 mL ultracentrifuge tube.
9. 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