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Title: Isolation and Validation of Podoplanin-positive Mesenchymal Stem Cell Subpopulations Using an Indirect Magnetic Bead Strategy

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, all done!**

Scope shots: 2.1.2, 2.3.3, 2.3.4, 2.8.2, 5.1.1, 6.1.2

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done!**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/14/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 27

Number of Shots: 58

Interviews

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Haofan Chen:** Our research focuses on isolating specific mesenchymal stem cell subsets through cost-effective magnetic bead sorting with high purity and viability.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Haofan Chen:** This protocol can be applied to any membrane protein using standard PE-conjugated antibodies.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Haofan Chen:** This approach enables researchers to study rare cell subpopulations with high purity and viability.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Haofan Chen:** The most important consideration in this protocol is gentle pipetting to maintain cell viability and remove unbound beads.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Haofan Chen:** Following this procedure, researchers can perform proliferation assays, flow cytometry, and differentiation studies.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

NOTE: SCREEN and SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Kathyayani Sridharan) for queries regarding lab media.

2. Preparation of a Single-Cell Suspension

Demonstrator: Haofan Chen

- 2.1. To begin, gather the required reagents [1]. Select mesenchymal stem cells or MSCs (M-S-C-S) from passages 3 to 5 that have reached 80 to 90 percent confluence [2]. Discard the culture medium [3].
 - 2.1.1. WIDE: Talent walking into the room with a tray of reagents and culture medium.
 - 2.1.2. SCOPE: 71365-2.1.2.mp4. 00:04–00:11
 - 2.1.3. Talent discarding the culture medium from the vessel.
- 2.2. Wash the cells twice with 10 milliliters of prewarmed PBS [1]. Gently rock the vessel [2] and discard the PBS [3].
 - 2.2.1. Talent adding 10 mL of prewarmed PBS to the cells.
 - 2.2.2. Talent gently rocking the vessel.
 - 2.2.3. Talent discarding the PBS.
- 2.3. Add 2 milliliters of 0.25 percent trypsin-EDTA [1] and incubate at 37 degrees Celsius for 3 to 5 minutes [2]. Under a microscope, observe the cells until they round up [3] and begin to detach [4].
 - 2.3.1. Talent adding 2 milliliters of 0.25 percent trypsin-EDTA to the cells.
 - 2.3.2. Talent placing the vessel in the incubator.
 - 2.3.3. SCOPE: 71365-2.3.3.mp4. 00:27–00:33
 - 2.3.4. SCOPE: 71365-2.3.4.mp4. 00:02–00:07
- 2.4. Add 4 milliliters of culture medium containing 16.5 percent FBS to neutralize the trypsin [1]. Gently pipette the cells to ensure complete detachment [2] and transfer the cell suspension to a 15-milliliter centrifuge tube [3].
 - 2.4.1. Talent adding 4 mL of culture medium to the vessel.
 - 2.4.2. Talent pipetting the cells in the vessel.
 - 2.4.3. Talent transferring the cell suspension to a 15mL centrifuge tube.
- 2.5. Centrifuge the cell suspension at 300 *g* for 3 minutes [1] and discard the supernatant [2].
 - 2.5.1. Talent placing the 15mL centrifuge tube in the centrifuge.

- 2.5.2. Talent discarding the supernatant from the centrifuge tube.
- 2.6. Resuspend the cell pellet in 1 milliliter of buffer [1].
 - 2.6.1. Talent adding 1mL of buffer to the cell pellet.
- 2.7. Centrifuge [1] and discard the supernatant as previously demonstrated [2].
 - 2.7.1. *Reuse 2.5.1.*
 - 2.7.2. *Reuse 2.5.2.*
- 2.8. Resuspend the cells with buffer to achieve a density of 100 million cells per milliliter [1]. Count the cells to determine the total cell number [2].
 - 2.8.1. Talent adding buffer to the cell pellet and resuspending the cells.
 - 2.8.2. SCOPE: 71365-2.8.2.mp4. 00:07–00:11.
- 2.9. Transfer the cell suspension to a 5-milliliter polystyrene round-bottom tube [1].
 - 2.9.1. Talent transferring the cell suspension into a 5mL polystyrene round-bottom tube.

3. Antibody and Magnetic Bead Labeling

- 3.1. Add 10 microliters of Fc gamma RII blocker (*F-C-gamma-R-two blocker*) per 10 million cells [1]. Mix gently and incubate at room temperature for 10 minutes [2].
 - 3.1.1. Talent adding Fc gamma RII blocker to the cell suspension tube.
 - 3.1.2. Talent mixing the cell suspension and placing it aside for incubation.
- 3.2. Add 5 microliters of phycoerythrin-conjugated or PE-conjugated (*P-E-conjugated*) anti-human podoplanin or PDPN (*P-D-P-N*) antibody per 10 million cells, following the manufacturer's recommended concentration [1]. Mix gently [2] and incubate at room temperature for 15 to 20 minutes protected from light [3].
 - 3.2.1. Talent adding the PE-conjugated anti-human PDPN antibody to the cell suspension tube.
 - 3.2.2. Talent mixing the cell suspension.
 - 3.2.3. Talent placing the wrapped tube on the laboratory bench for incubation at room temperature.
- 3.3. Add 10 microliters of Selection Cocktail per 10 million cells [1]. Mix gently and incubate at room temperature for 15 minutes [2].
 - 3.3.1. Talent adding the Selection Cocktail to the cell suspension tube.

3.3.2. *Reuse 3.1.2*

3.4. Use a vortex mixer to mix the magnetic beads vial, vigorously for 30 seconds [1]. Add 5 microliters of magnetic beads per 10 million cells [2]. Mix gently and incubate at room temperature for 10 minutes [3].

3.4.1. Talent vortexing the magnetic beads vial vigorously.

3.4.2. Talent adding the magnetic beads to the cell suspension tube.

3.4.3. *Reuse 3.1.2*

4. Magnetic Cell Separation

4.1. Place the 5-milliliter polystyrene round-bottom tube containing the labeled cell sample in the magnetic separation stand [1].

4.1.1. Talent placing the 5mL polystyrene round-bottom tube containing the labeled cell sample into the magnetic separation stand.

4.2. Add buffer to the tube to bring the total volume to 2.5 to 3 milliliters and incubate at room temperature for 5 minutes [1].

4.2.1. Talent adding buffer to the tube in the magnetic separation stand and keeping it aside for incubation.

4.3. Holding the magnet and tube together as one unit, invert them to pour the supernatant into a new collection tube [1]. Place the collected PDPN-negative cell fraction on ice [2].

4.3.1. Talent inverting the magnetic separation stand with the tube and pouring the supernatant into a new collection tube.

4.3.2. Talent placing the collection tube containing the PDPN-negative cell fraction on ice.

4.4. Repeat the magnetic separation procedure 3 times as previously demonstrated, pouring the supernatant into the same PDPN-negative fraction collection tube after each separation [1].

4.4.1. Talent inverting the magnetic separation stand with the tube and pouring the supernatant into the existing PDPN-negative fraction collection tube.

4.5. After the final wash, remove the tube containing the magnetically bound PDPN-positive cell fraction from the magnetic separation stand [1].

4.5.1. Talent removing the tube from the magnetic separation stand.

4.6. Add 3 milliliters of buffer to resuspend the positive cells [1]. Transfer the positive fraction cell suspension to a fresh labeled tube [2].

4.6.1. Talent adding buffer to the tube and resuspending the positive cells.

4.6.2. Talent transferring the cell suspension into a fresh labeled tube.

5. Cell Counting and Culture Preparation

5.1. Count the cells from both the positive and negative fraction tubes [1]. Record the cell yield for each fraction [2].

5.1.1. SCOPE: 71365-5.1.1.mp4. 00:02–00:06.

5.1.2. Talent recording the cell yield for each fraction in a lab notebook.

5.2. Centrifuge the positive fraction cell suspension at 300 *g* for 3 minutes [1] and discard the supernatant [2].

5.2.1. Talent placing the positive fraction cell suspension tube in the centrifuge.

5.2.2. Talent discarding the supernatant from the tube.

5.3. Resuspend the cell pellet in MEM (*M-E-M*) Alpha culture medium containing 16.5 percent FBS [1]. Adjust the cell density as needed [2] and seed the cells into culture plates or flasks [3].

5.3.1. Talent resuspending the cell pellet in MEM Alpha culture medium.

5.3.2. Talent adding culture medium to the cell suspension to obtain the desired cell density

5.3.3. Talent adding the cells into culture plates or flasks.

5.4. Incubate the cells at 37 degrees Celsius in a 5 percent carbon dioxide incubator [1].

5.4.1. Talent placing the culture plates or flasks into the carbon dioxide incubator.

6. Purity and Functional Validation of Isolated Cells

6.1. After counting the cells, seed them onto a 100-millimeter culture dish at a density of 3 million cells per dish in 12 milliliters of culture medium [1]. Twenty-four hours after seeding, observe cell attachment [2]. Replace the culture medium with fresh medium to remove residual magnetic beads and non-adherent cells [3].

6.1.1. Talent adding the cells into a 100mm culture dish containing 12mL of culture medium.

6.1.2. SCOPE: 71365-6.1.2(1).mp4. 00:12–00:19.

6.1.3. Talent removing the culture medium and adding fresh medium to the culture dish.

6.2. Harvest the cells by trypsinization [1]. Gently pipette the cell suspension up and down 10 to 15 times to dislodge and remove magnetic beads and residual antibodies [2].

- 6.2.1. Talent adding trypsin to the cells.
- 6.2.2. Talent pipetting the cell suspension up and down to remove magnetic beads and residual antibodies.
- 6.3. Assess the percentage of PDPN-PE positive cells by flow cytometry [1].
 - 6.3.1. SCREEN: 71365_screenshot_1.mp4. 00:03–00:07. *Video Editor: Please emphasize the value 95.8*
- 6.4. Collect cells during the logarithmic growth phase [1]. Seed the cells into a 96-well plate [2]. At different time points, add Cell Counting Kit-8 or CCK-8 (*C-C-K-eight*) reagent [3-TXT] and measure absorbance according to the manufacturer's instructions [4]. Tabulate the pre-sort and post-sort data for different timepoints in **GraphPad Prism** software [5], then click on **Data** to plot the proliferation curve [6]. **Note: The narration and numbers are adjusted for the added SCREEN shot.**
 - 6.4.1. Talent collecting cells during the logarithmic growth phase.
 - 6.4.2. Talent adding the cells into a 96-well plate.
 - 6.4.3. Talent adding Cell Counting Kit-8 reagent to the wells of the plate. **TXT: Time points: 0 h, 24 h, 48 h, 72 h**
 - 6.4.4. Talent loading the 96-well plate into a microplate reader for absorbance measurement
 - 6.4.5. SCREEN: 71365_screenshot_2.mp4. 00:02–00:07
 - 6.4.6. SCREEN: 71365_screenshot_2.mp4. 00:08–00:12

Results

7. Results

- 7.1. Following Fc receptor blocking, antibody labeling, and magnetic separation, the final cell count was approximately 3.5 million cells for the PDPN-positive fraction [1] and 6.4 million cells for the PDPN-negative fraction [2].
 - 7.1.1. LAB MEDIA: Figure 2B. *Video editor: Emphasize the red bar labeled "PDPN+".*
 - 7.1.2. LAB MEDIA: Figure 2B. *Video editor: Emphasize the blue bar labeled "PDPN-".*
- 7.2. Before magnetic sorting, the frequency of PDPN-positive cells in the starting cell population was 38.7% [1].
 - 7.2.1. LAB MEDIA: Supplemental Figure S1A. *Video editor: Emphasize the gated box with label PDPN+ 38.7 on the flow cytometry plot.*
- 7.3. After sorting, the positive fraction contained 97.9% PDPN-PE-positive cells [1].
 - 7.3.1. LAB MEDIA: Figure 2C. *Video editor: Emphasize the gated box with label PDPN+ 97.9 on the flow cytometry plot.*
- 7.4. Flow cytometry analysis of the negative fraction revealed that 4.30% of PDPN-positive cells remained [1], indicating efficient depletion of PDPN-positive cells from the negative fraction [2].
 - 7.4.1. LAB MEDIA: Supplemental Figure S1B. *Video editor: Emphasize the gated box with label PDPN+ 4.30 on the flow cytometry plot.*
 - 7.4.2. LAB MEDIA: Supplemental Figure S1B.
- 7.5. The proliferation curve of PDPN-positive cells over 3 consecutive days of culture showed no significant difference compared to unsorted MSCs [1], indicating that the sorting strategy did not cause a detectable reduction in CCK-8-based proliferation or metabolic activity compared to unsorted MSCs [2].
 - 7.5.1. LAB MEDIA: Figure 2D. *Video editor: Emphasize the red and blue lines and the "ns" above the data points.*

Pronunciation Guide:

1. Mesenchymal
Pronunciation link: <https://www.merriam-webster.com/dictionary/mesenchymal>
IPA: /ˌmez.ənˈkaɪ.məl/
Phonetic Spelling: mez·uhn·KYE·muhl

2. Confluence
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /'kɑ:n.flu.əns/
Phonetic Spelling: KAHN·floo·uhns
3. Phosphate-buffered saline (PBS)
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /'fɑ:s.feɪt/; /'bʌf.əd/; /'seɪ.laɪn/
Phonetic Spelling: FOSS·fayt; BUF·erd; SAY·line
4. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪp.sɪn/
Phonetic Spelling: TRIP·sin
5. EDTA
Pronunciation link: <https://www.howtopronounce.com/edta>
IPA: /,i:.di:.ti:'eɪ/
Phonetic Spelling: ee·dee·tee·AY
6. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪŋ.kjə.beɪt/
Phonetic Spelling: ING·kyuh·bayt
7. Neutralize
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralize>
IPA: /'nu:.trə.laɪz/, /'nju:.trə.laɪz/
Phonetic Spelling: NOO·truh·lyze; NYOO·truh·lyze
8. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trə.fju:dʒ/
Phonetic Spelling: SEN·truh·fyooj
9. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,ri:.sə'spend/
Phonetic Spelling: ree·suh·SPEND
10. Polystyrene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polystyrene>
IPA: /,pɑ:.li'staɪ.rɪn/
Phonetic Spelling: pol·ee·STYE·reen
11. Fc gamma RII
Pronunciation link: No confirmed link found
IPA: /,ef'si: 'gæm.ə ɑ:r tu:/

Phonetic Spelling: ef·SEE GAM·uh ar TWO

12. Phycoerythrin

Pronunciation link: <https://www.howtopronounce.com/phycoerythrin>

IPA: /ˈfaɪ.koʊ.ɪˈrɪθ.rɪn/

Phonetic Spelling: FYE·koh·ih·RITH·rin

13. Conjugated

Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugated>

IPA: /ˈkɑːn.dʒəˌgeɪ.tɪd/

Phonetic Spelling: KON·juh·gay·tid

14. Podoplanin (PDPN)

Pronunciation link: <https://www.howtopronounce.com/podoplanin>

IPA: /ˌpɒʊ.doʊˈplæn.ɪn/

Phonetic Spelling: poh·doh·PLAN·in

15. Vortex

Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>

IPA: /ˈvɔːr.teks/

Phonetic Spelling: VOR·teks

16. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsuː.pəˈnei.tənt/

Phonetic Spelling: soo·per·NAY·tuhnt

17. Logarithmic

Pronunciation link: <https://www.merriam-webster.com/dictionary/logarithmic>

IPA: /ˌlɒːɡəˈrɪð.mɪk/

Phonetic Spelling: law·guh·RITH·mik

18. Adherent

Pronunciation link: <https://www.merriam-webster.com/dictionary/adherent>

IPA: /ədˈhɪr.ənt/

Phonetic Spelling: ad·HEER·uhnt

19. Trypsinization

Pronunciation link: <https://www.howtopronounce.com/trypsinization>

IPA: /ˌtrɪp.sɪ.nəˈzeɪ.ʃən/

Phonetic Spelling: trip·sin·uh·ZAY·shuhn

20. Flow cytometry

Pronunciation link: <https://www.merriam-webster.com/dictionary/cytometry>

IPA: /floʊ saɪˈtɑː.mə.tri/

Phonetic Spelling: floh sy·TOM·uh·tree

21. Absorbance

Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>

IPA: /əb'zɔːr.bəns/

Phonetic Spelling: ub·ZOR·buhns

22. GraphPad Prism

Pronunciation link: <https://www.graphpad.com/>

IPA: /græf.pæd 'prɪz.əm/

Phonetic Spelling: graf·pad PRIZ·um

23. Proliferation

Pronunciation link: <https://www.merriam-webster.com/dictionary/proliferation>

IPA: /prə'lif.ə'reɪ.jən/

Phonetic Spelling: pruh·LIF·uh·RAY·shuhn

24. MEM Alpha

Pronunciation link: No confirmed link found

IPA: /,em.i:'em 'æl.fə/

Phonetic Spelling: em·ee·EM AL·fuh