

TheraPEAK® MSC-VIVO® Medium

Recommended instructions for use

Contents:

Section	Description	Page
I	MSC media preparation	1
II	MSC thawing and 2D plating protocol	1
III	MSC 3D spinner flask culture	2
IV	Ordering Information	3
V	Product Use Statement	3

I. TheraPEAK® MSC-VIVO® Media preparation

For 2D growth:

1. Warm base medium at 37°C for 5 minutes.
2. Thaw the 2D supplement (5 mL volume) at room temperature.
3. Once thawed, add the entire contents of the 2D supplement to 1 L of pre-warmed base medium.
4. Mix gently to ensure complete incorporation of the supplement.

For 3D growth and exosome production:

1. Warm base medium at 37°C for 5 minutes
2. Thaw the 3D supplement (5 mL volume) at room temperature.
3. Once thawed, add the entire contents of the 3D supplement to 1 L of pre-warmed base medium.
4. Mix gently to ensure complete incorporation of the supplement.

NOTE: Store prepared medium at 4°C and protected from light. Warm only the necessary volume of medium to 37°C prior to use. Ensure all media and reagents are pre-warmed to 37°C before use. Be gentle during resuspension and pipetting to prevent cell stress or damage.

II. MSC thawing and 2D plating protocol

Thawing MSC cells from liquid nitrogen:

1. Retrieve a vial of cells from the frozen storage.
2. Thaw the cells in a water bath at 37°C until only a small ice crystal remains (roughly 2 – 3 minutes). Make sure the top of the vial does not directly touch the water.
3. After thawing, spray the vial with 70% isopropanol to disinfect the exterior.

Cell resuspension:

1. Prepare a 15 mL conical tube by adding 4 mL of pre-warmed complete medium.
2. Using a 1000 µL pipette, resuspend the thawed cells gently in the warm media. (Do not pipette up and down; instead, gently add the thawed cells to the medium).

Removal of cryoprotectant and cell assessment:

1. Centrifuge the cell suspension at 300 x g for 5 minutes to pellet the cells.
2. Gently aspirate the supernatant to avoid disturbing the pellet. Resuspend the cell pellet in 1 mL of pre-warmed medium.
3. Mix the cells by gently pipetting up and down 3 – 4 times using a 1 mL pipette to ensure a uniform distribution of the cells.
4. Perform a cell count and viability assessment to ensure that the starting cell population is appropriate for use.

Plating cells for 2D culture:

Day 1:

1. Plate 3000 cells/cm² in 5 mL of pre-warmed medium in a T25 CellBind® Corning® flask or other appropriate scale tissue culture treated flask.
2. Incubate the flask at 37°C in a humidified, 5% CO₂ incubator for 72 hours.

Day 3:

1. Completely remove and replace the medium. Perform a microscopic observation of the culture to confirm cell attachment and proliferation.
2. Monitor and observe the cell growth daily and harvest the cells when the flask reaches ~90% confluency which should occur roughly between day 5 and 6.

Cells can be passed into a new tissue culture treated vessel at the same seeding density (3000 cells/cm² in 5 mL of pre-warmed medium) for further expansion **OR** proceed to downstream analysis.

3. Perform downstream analytical testing, as desired. Recommended analyses include:
 - Cell count and viability
 - Cell surface antigen expression
 - Differentiation potential to osteocytes, chondrocytes and / or adipocytes
 - Cell functionality includes wound healing, cytokine secretion profile, etc.

III. MSC 3D spinner flask culture

Day 0 – Spinner flask preparation:

1. In a biosafety cabinet, weigh 1.05 g of SoloHill® collagen-coated microcarrier beads or Synthemax® II Microcarriers to provide a surface area of 360 cm².
2. In a biosafety cabinet, open a sterile spinner flask and add 25 mL of complete medium gently to the side of the flask tube wall to avoid disrupting the beads.
3. Equilibrate the beads in the medium while thawing and preparing the cells as described in **Thawing MSC Cells from Liquid Nitrogen**.

4. Seed 1.08 x 10⁶ cells into the medium to ensure a uniform distribution of cells and allow for a seeding density of 3000 cells/cm².
5. Place the spinner flask on a magnetic stirrer inside the humidified, 5% CO₂ incubator set to a stir speed of 25 rpm.

Day 1 – Media addition:

1. After 24 hours, carefully add 50 mL of warm medium to the side of the spinner flask. Avoid disturbing the beads.
2. Increase spinner speed to 45 rpm and place the spinner flask back in the incubator.
3. Continue incubating culture for an additional 4 days.

Day 5 – Medium change:

1. In a biosafety cabinet, gently aspirate approximately 90% of the medium (~60 – 65 mL) using a sterile aspirator.
2. Add fresh pre-warmed medium (~60 – 65 mL) and return the spinner flask to the spinner platform (set to 45 rpm) to incubate for an additional 5 days.

Day 10 – Cell harvest, EV collection, and cell detachment:

1. Check for cell clumps in the spinner flask as an indicator of successful proliferation.
2. Using a 1 mL wide bore pipette, gently remove some of the clumps of cells and place on a slide for image capture (recommend min. 4x magnification).
3. Allow the beads to settle by tilting the spinner flask to approximately a 45° angle.
4. Using a 50 mL serological pipette, aseptically aspirate 50 mL of medium for EV particle analysis, leaving approximately 5 mL of medium in the flask.
5. Add 5 mL of TrypLE® Reagent gently for a 1:1 ratio with the remaining medium.
6. Transfer contents of the spinner flask to a 50 mL conical tube.
7. Add an additional 5 mL of TrypLE® to the flask to ensure that all of the cells and beads are collected. The total volume in the 50 mL conical tube should now be approximately 15 mL.

Incubate the bead and cell suspension at 37°C for 3 minutes to allow cell detachment.

8. Gently mix by pipetting up and down 3 – 4 times. Repeat this step 2 – 3 times, if necessary, to fully dissociate the cells.
9. Stop the TrypLE® reaction by adding 10 mL of complete medium.
10. Transfer the contents to a fresh 50 mL conical by filtering through a 70-micron strainer.
11. Flush any remaining cells through the strainer by adding 5 mL of medium.

The total volume in the 50 mL conical tube should be approximately 30 mL.

12. Remove a sample of the cell suspension for counting and viability assessment.
13. Centrifuge the cell suspension at 300 x g for 5 minutes to pellet the cells and remove the TrypLE® detachment reagent.
14. Resuspend the cells in fresh medium and perform analytical assessment of the cells as desired.

Supernatant centrifugation for downstream EV analysis

1. Centrifuge the collected supernatant for particle analysis as follows:
 - 1st spin: Centrifuge at 300 x g for 10 minutes. Transfer the supernatant to a fresh tube.
 - 2nd spin: Centrifuge at 1200 x g for 10 minutes. Transfer the supernatant to a fresh tube.
 - 3rd spin: Centrifuge at 10,000 x g for 30 minutes. Transfer the supernatant to a fresh tube.
2. Use the supernatant immediately for analysis or store at -80°C for later use.
3. Perform EV analyses as desired. Suggested measurements include:
 - Particle count via ZetaView® Evolution as per Particle Metrix recommendations.
 - EV quality assessment using NanoFCM Single-Molecule Nanoanalyzer as per vendor's recommendations to assess surface markers and intact EVs.
 - EV functionality assays including, but not limited to, wound healing, migration enhancement or other relevant assays.

IV. Ordering information

Kit type	Kit components	Catalog no.
TheraPEAK® MSC-VIVO® for 2D applications	TheraPEAK® 2D Base Medium, 1L TheraPEAK® 2D Supplement, 5mL	BP18-972
TheraPEAK® MSC-VIVO® for 3D applications	TheraPEAK® 3D Base Medium ,1L TheraPEAK® 3D Supplement, 5mL	BP18-973

V. Product use statement:

All trademarks belong to Lonza Group Ltd or its affiliates ("Lonza Group"), and are registered in the USA, EU and/or CH or used in common law, or belong to their respective third-party owners and are only being used for informational purposes. All third-party copyrights have been reproduced with permission from their owners. The information contained herein is believed to be correct and corresponds to the latest state of scientific and technical knowledge. However, no warranty is made, either expressed or implied, regarding its accuracy or the results to be obtained from the use of such information and no warranty is expressed or implied concerning the use of these products. The buyer assumes all risks of use and/or handling. Any user must make his own determination and satisfy himself that the products supplied by Lonza Group and the information and recommendations given by Lonza Group are (i) suitable for intended process or purpose, (ii) in compliance with all applicable laws, including all environmental, health and safety regulations, and (iii) will not infringe any third party's intellectual property rights. The user bears the sole responsibility for determining the existence of any such third-party rights, as well as obtaining any necessary licenses and approvals. For more details: <http://www.lonza.com/legal>.

© 2026 Lonza. CT-OP012 07/26. All rights reserved.