Tribo™ MSC Medium (TBS8021)

Product Overview

Tribo™ MSC Medium is a chemically defined medium for growth and expansion of human mesenchymal stem cells (MSCs) derived from bone marrow. It does not contain animal-derived components, which eliminate concerns of lot-to-lot variations and potential animal-borne pathogens. Using MSC Medium, human MSCs can be quickly and optimally expanded while maintaining their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages.

MSC Medium further offers the convenience of no plate-coating, which means that you can use standard tissue culture plates or flasks right out of their package – no more expensive coating materials and time-consuming procedure.

Content and Storage of the Product

MSC Medium (Catalog No. TBS8021) contains two components:

Component	Size	Storage	Shelf Life
MSC Medium Basal Medium	1 x 450mL	2 to 8°C. Protect from light	6 months
MSC Medium Supplement	1 x 50mL	-20°C or below. Protect from light	6 months

Medium Preparation

1. Thaw MSC Medium Supplement. It is recommended to thaw overnight at 2 to 8°C. Thawed material can be aliquoted and again stored at -20°C or below, but further freeze-thaw cycles should be avoided.
2. To make 100 mL MSC Medium complete medium, aseptically add 10 mL MSC Medium Supplement to 90 mL MSC Medium Basal Medium. Then aseptically add 1 mL L-Glutamine (200 mM, or 100x) to the complete medium.

Antibiotics such as the Pen-Strep solution can also be added to the complete medium if so desired. We Recommend the final concentrations be 25 units/mL Penicillin and 25 microgram/mL Streptomycin. These concentrations are 4 times lower than those used in traditional, serum-containing media. The reason more antibiotics have to be used in serum-containing media is that serum contains elements that absorb antibiotics and decrease their availability.

Once made, MSC Medium (basal, supplement and L-glutamine) is stable for 1 month when stored in the dark at 2 to 8°C.

Protocols for culturing human MSCs in MSC Medium

I. **Recovery of Cryopreserved Human MSCs:** Frozen human MSCs, regardless of what medium was used to grow or freeze them before, can be easily and quickly adapted into MSC Medium. A one-step transition into MSC Medium as outlined below is generally sufficient for cells that have been grown and frozen in serum-containing medium or serum-free media.

1. Rapidly thaw frozen vial of cells in a 37°C water bath.
2. Transfer the cells into a 15 mL conical tube.
3. Add 10mL of pre-warmed (37°C) MSC Medium in a drop-wise manner while gently swirling the tube.
4. Transfer the entire contents of the conical tube into a tissue culture flask or multiple wells of a tissue culture plate. Alternatively, cells can also be centrifuged at 250 x g (~1200 rpm) for 10 minutes, resuspended in MSC Medium, and then plated.
5. Incubate at 36 to 38°C in a humidified atmosphere containing 4 to 6% CO_2.
6. Change media after 24 hrs.

II. **Subculturing MSCs in MSC Medium**: Coating of cell culture vessels is NOT necessary when culturing human MSCs in MSC Medium. Standard tissue culture treated vessels (e.g. BD Falcon™ or Corning™) can support optimal cell attachment and spreading when cells are cultured in MSC Medium. If otherwise desired, an 1 hr coating with Fibronectin (Cat# **TBS8022**) may be performed, which slightly increases the rate of cell growth by 10-20%. MSCs grown in serum-containing or serum-free media can be quickly and easily adapted into MSC Medium. In most cases, a one-step transition into MSC Medium is sufficient. If so desired, step-wise adaptation with gradual increase in the amount of MSC Medium (e.g. 20%, 40%, etc) can also be performed.

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1. Visually inspect the stock culture (growing in MSC Medium or other medium formulation) under the microscope and confirm that the cells are ready to be sub-passaged. It is recommended that the input cells prior to subculture have a confluence of 60 to 80%, viability of at least 90% and the growth rate in mid-logarithmic phase.
2. Pre-warm 0.25% Trypsin/EDTA (e.g. GIBCO 25200) or TrypLE™ Express (GIBCO 12604) and MSC Medium complete medium to 37°C before use.
3. Remove spent (old) medium from the flask using a pipette and discard.
4. Wash tissue culture surface with DPBS, remove and discard.
5. Add the 0.25% Trypsin/EDTA solution or TrypLE™ Express to the flask, tilt flask to cover all the cells. We recommend detaching the cells at room temperature.

	Individual well of a 6-well plate (~10 cm^2)	T25 flask (25 cm^2)
Volume of Trypsin or TrypLE Express	0.6 mL	1.5 mL

6. Observe the cells under a microscope. When cells start to detach, gently tap the side of the vessel to help loosen the remaining cells. The time required for the cells to detach should be 1 to 3 minutes if the cells have been cultured in MSC Medium. Cells grown in serum-containing media will require longer incubation time to detach.

   Once all the cells have detached, proceed quickly to the following step. Do not leave cells in Trypsin or TrypLE™ Express for an extended amount of time after the cells have detached, as this will adversely affect the growth of MSCs.
7. Upon cell detachment, add enough DPBS to cover the surface area. Collect the cell suspension in a sterile 15 mL conical tube. Tap flask firmly or pipet the suspension to break cell clumps if necessary.

   Optional: If 0.25% Trypsin/EDTA solution is used to detach the cells, it is desirable to use the Soybean Trypsin Inhibitor to neutralize trypsin. This is necessary if the washing steps (steps 7 and 8) cannot be processed quickly (e.g. longer than 20 minutes). [This is because serum-free media formulations like MSC Medium do not contain antitrypsin, a component found in animal serum. Unless removed quickly by centrifugation, trypsin can damage the cells and affect growth.] As suggested by its manufacture, it is not necessary to neutralize TrypLE Express.
8. Centrifuge cells at 1200 rpm (250 x g) for 10 minutes.
9. Aspire and discard as much supernatant as possible. Resuspend cells in either DPBS or spent medium, and centrifugate again. If trypsin inhibitors are used, skip step 9 and proceed to step 10.
10. Aspire the supernatant and resuspend cells in pre-warmed MSC Medium. Take a small aliquot from the cell suspension for cell counting.
11. Place **$5x10^3$ cells/cm^2** to each flask. Tilt the vessel a few times to ensure even distribution of cell suspension.

	Individual well of a 6-well plate (~10 cm^2)	T25 flask (25 cm^2)
Seeding cell number	5 x 10^4	1.25 10^5

12. Incubate at 36 to 38°C in a humidified atmosphere of 4 to 6% CO_2.
13. Replace culture medium every 2 days with fresh, pre-warmed MSC Medium.

III. Cryopreservation of Cells in MSC Medium

1. Prepare cryopreservation solution by supplementing MSC Medium Basal Medium with 10% MSC Medium Supplement and 10% Dimethyl Sulfoxide (DMSO).
2. Pellet cells by centrifugation, gently resuspend cells in cryopreservation solution to 1.0x10^6cells/mL, and transfer to cryovials.
3. Place cryovials in a freezing container (e.g. Nalgen 5100-0001) and place in a -70°C freezer overnight.
4. Transfer cryovials to liquid nitrogen for long-term storage.

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