



# Culturing Human Mesenchymal Stem Cells using MSC NutriStem® Basal Medium supplemented with PLTMax® Human Platelet Lysate

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## Required Materials

Reagent	Source	Cat. No.
MSC NutriStem® Basal Medium	BI-USA	05-200-1A
PLTMax® Human Platelet Lysate	BI-USA	PLTMAX100
DPBS, no calcium, no magnesium	BI-USA	02-023-1
Recombinant Trypsin Solution or Recombinant Trypsin-EDTA Solution	BI-USA	03-078-1 03-079-1
Heparin, or other anti-coagulant	-	-

## Introduction

Platelets play an important role in tissue repair and wound healing within the body. The growth factors and cytokines naturally found in platelet-rich plasma are contained within extracted platelet lysate solution. Human platelet lysate is an effective, high-protein supplement for human cell culture and expansion, including mesenchymal stem/stromal cells (MSC)<sup>1</sup>. In both research and clinical applications, human platelet lysate is often used as an alternative to animal sera, such as fetal bovine serum (FBS), in order to minimize the risk of xenogenic immune reactions or transmitting prions and zoonotic diseases<sup>2</sup>.

**PLTMax® Human Platelet Lysate** has proven to be a superior alternative to FBS in MSC culture systems. PLTMax was the first commercially available human platelet lysate for research and clinical use. PLTMax is manufactured to control lot-to-lot variability from a thoroughly-screened donor pool. Clinical-grade PLTMax has been used to grow cells to treat hundreds of patients and has been used in over 30 clinical trials worldwide.

**MSC NutriStem® Medium** is a defined, xeno-free medium, recognized as an optimal medium to culture human MSCs. Pairing PLTMax with MSC NutriStem Basal Medium creates an excellent medium for expansion of MSCs in an animal serum-free environment<sup>3</sup>. Additionally, this combination is suitable for translational applications, as both components are produced following cGMP and regulatory guidelines for clinical use, including individual drug master files (DMF) accepted by the FDA.

The following protocol outlines the routine culture procedure for expanding human MSCs in MSC NutriStem Basal Medium supplemented with 5% PLTMax, and can be used as a guideline to establish optimal culture parameters for each unique MSC line or application.

## Reagent Storage, Handling, and Notes

### PLTMax® Human Platelet Lysate:

- Thaw frozen PLTMax in a 37°C water bath, protected from light. Mix gently but thoroughly once thawed.
- If not using immediately, prepare single-use aliquots from the stock solution, and re-freeze at -20°C.
- Avoid exposing PLTMax to repeated temperature changes or freeze/thaw cycles. Long-term storage of PLTMax at 4°C is not recommended, as this can increase the formation of precipitates and/or coagulation occurring in the product.
- Due to the presence of certain plasma components and coagulation factors, PLTMax requires the supplementation of an anti-coagulant, such as heparin, to a final concentration of 2 Units/mL in the cell culture media in order to prevent clot formation.

**Note:** PLTGold® Human Platelet Lysate can be substituted for PLTMax if a xeno-free solution is required.

### MSC NutriStem® Basal Medium:

- MSC NutriStem Basal Medium must be stored at 4°C and protected from light.
- MSC NutriStem Basal Medium contains stable L-glutamine; no additional L-glutamine is necessary.

### MSC Medium Preparation and Storage:

#### To prepare 100 mL of complete MSC medium:

- Aseptically add 5.5 mL of PLTMax to 100 mL of MSC NutriStem Basal Medium.
- Add heparin to a final concentration of 2 U/mL.

#### To prepare 500 mL of complete MSC medium:

- Aseptically add 26 mL of PLTMax to 500 mL of MSC NutriStem Basal Medium.
- Add heparin to a final concentration of 2 U/mL.

Store complete MSC NutriStem Basal Medium supplemented with PLTMax at 4°C, protected from light, for up to 2 weeks.

## Methods

### Notes:

- In general, cells do not require an extended adaptation phase when transitioning to MSC NutriStem Basal Medium supplemented with PLTMax as the culture medium.
- Cells can be directly seeded in MSC NutriStem Basal Medium supplemented with PLTMax upon a thaw.
- Cell seeding should be performed following the general guidelines for the specific cell type. Typically, human MSCs are plated at approximately  $2 \times 10^3$  to  $5 \times 10^3$  cells per  $\text{cm}^2$ .
- Prior to feeding or passaging, warm only the amount of medium that will be used that day. Discard any excess warmed media at the end of each day.

### Culture and Maintenance of MSCs in MSC NutriStem Basal Medium Supplemented with PLTMax

For best results, MSCs should be passaged when the cell confluency reaches 60% to 70%. Do not allow MSCs to overgrow in culture (over 70% 80% confluency). For many MSC lines, the cells grow rapidly in this medium, and passaging should be performed every 2 to 4 days. Perform a complete medium exchange every other day while cells are being maintained in culture.

**For clinical applications,** a clinical-grade heparin, such as Sodium Heparin (USP) for IV use is recommended.

**Reminder:**  
Feed cells every other day while maintaining the culture.

**Recombinant Trypsin-EDTA Solution** can be used if cells are over-confluent or difficult to detach with Recombinant Trypsin Solution.

**Because most cells proliferate rapidly** in MSC NutriStem and PLTMax, do not plate more than  $5 \times 10^3$  cells/cm<sup>2</sup> in the new culture vessel to avoid overconfluence prior to passaging.

## Passaging

1. Briefly warm a sufficient amount of complete MSC NutriStem Basal Medium supplemented with PLTMax at 37°C.
2. Using a vacuum aspirator and sterile aspirator pipette, remove the supernatant from the culture vessel to be passaged.
3. Add a sufficient volume of DPBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>) to wash the culture surface. Use approximately 2 mL of DPBS per 10 cm<sup>2</sup> culture surface area.
4. Gently rock the culture vessel to wash the cells, and aspirate the DPBS.
5. To detach the cells from the culture surface, add a sufficient volume of Recombinant Trypsin Solution to cover the cell culture surface, and incubate cells at room temperature or 37°C for 2 to 4 minutes. Tap the vessel periodically to expedite cell detachment and monitor the progress.
6. Observe the cells under a microscope. If less than 90% of the cells are detached from the surface, continue incubating and observe at 1-minute intervals to check for complete detachment of the cells.

**Note:** Incubation times will vary between cell lines and confluency. Begin checking the culture after 2 minutes. Do not over-incubate the culture, as MSCs can be sensitive to enzymatic stress.

7. Once the cells are detached from the culture surface, quench the action of the trypsin by adding a volume of pre-warmed MSC NutriStem Basal Medium supplemented with PLTMax that is 4 times the volume of the Recombinant Trypsin Solution used.
8. Collect the cell suspension and transfer to a centrifuge tube. If needed, rinse the culture vessel with additional media to collect and transfer any remaining cells.
9. Centrifuge at 200 x g for 5 minutes at room temperature.
10. Remove the supernatant and suspend the cell pellet in 5 mL of MSC NutriStem Basal Medium supplemented with PLTMax.
11. Perform a cell count and calculate viability, concentration (cells/mL), and total cell number.
12. Plate the cells following at approximately  $2 \times 10^3$  to  $5 \times 10^3$  cells per cm<sup>2</sup>, or by following the general guidelines for the specific cell type.

**Note:** Depending on the cell count and culture vessel(s) to be plated, additional MSC NutriStem Basal Medium supplemented with PLTMax can be added to reach the total volume required.

13. Incubate the cells at 37°C and 5% CO<sub>2</sub>.
14. Observe cells daily to monitor cell health, proliferation, and confluence. Perform a complete medium change every other day as needed between passages.

## References:

1. Mohammadi, S. et al. 2016. Human Platelet Lysate as a Xeno Free Alternative of Fetal Bovine Serum for the In Vitro Expansion of Human Mesenchymal Stromal Cells. *IJHOSCR* 10(3), 161 – 171.
2. Astori, G. et al. 2016. Platelet lysate as a substitute for animal serum for the ex-vivo expansion of mesenchymal stem/stromal cells: present and future. *Stem Cell Research & Therapy* 7:93.
3. Alonso-Camino, V. and Kent, L. T. 2017. Poster: Expansion of MSCs for Translational Medicine using MSC NutriStem® Basal Medium and PLTMax® Human Platelet Lysate. Retrieved from [www.bioindusa.com](http://www.bioindusa.com)

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