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Application Note

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Passaging MSC Using NutriStem® XF Medium and Human Platelet Lysate

Clinically Compatible Xeno-Free, Animal Serum-Free Culture System

Introduction

Mesenchymal stem|stromal cells (MSC) are promising tools in the field of regenerative medicine and the creation of cellular therapies. When expanding MSC in vitro for translational use, a clinically relevant and cGMP-compliant culture system that generates large numbers of healthy, proliferative MSC is vital. Human MSC from multiple sources cultured in MSC NutriStem® XF Medium supplemented with human platelet lysate show exceptional proliferation and allow for long-term MSC culture and rapid expansion in a clinically compatible environment. Traditional MSC culture protocols often rely on non-optimal classical basal media, such as alpha-MEM or DMEM, supplemented with human or animal serum, most often fetal bovine serum (FBS). It is known, however, that these media are not optimized for MSC, and are not suited for translational applications.

Sartorius' Advanced Therapies' MSC NutriStem® XF Medium is a cGMP-manufactured, defined, serum-free, xeno-free human MSC culture medium, composed of the highly optimized basal medium and supplement mix specifically designed for human MSC culture. MSC NutriStem® XF Medium is currently being used in multiple clinical trials worldwide, and is adaptable for culturing a diverse range of cell populations in variety of applications[1], including stem cell-derived exosome studies[2].

Human platelet lysate is an effective, high-protein supplement for human cell culture and expansion, including MSC[3]. Serum constitutes a risk when MSC are produced for clinical applications and increases the complexity of downstream processing[4]. In both research and clinical applications, human platelet lysate is often used as an alternative to animal sera such as FBS, in order to minimize the risk of xenogenic immune reactions or transmitting prions and zoonotic diseases[5].

PLTGold® Human Platelet Lysate is a xeno-free, animal serum-free supplement, proven to show superior growth and proliferation over FBS and human serum in MSC culture systems. PLTGold® is unfractionated (not fibrinogen-depleted) platelet lysate, containing all of the growth factors and proteins necessary for cell growth, and is manufactured to control lot-to-lot variability from a thoroughly-screened clinical donor pool.

Pairing MSC NutriStem® XF Medium with PLTGold® Human Platelet Lysate creates and excellent culture system for the expansion of MSC in a completely xeno-free, animal serum-free environment. This MSC culture system does not require any attachment substrate for standard 2D culture, and is flexible to support multiple culture formats requiring enhanced cell proliferation and culture longevity.

Therapeutic doses of autologous or allogenic MSC often require the move from standard 2D to 3D bioreactor systems to enable mass cell expansion. The MSC NutriStem® XF Medium and PLTGold® Human Platelet Lysate culture system is suitable for scale-up and translational applications, as healthy MSCs tend to proliferate rapidly in long-term culture. Additionally, all media components are produced following cGMP and regulatory guidelines for clinical use, including individual drug master files (DMF) accepted by the FDA.

Required Materials

Reagent	Cat. No.
MSC NutriStem® XF Medium	05-200-1A 05-201-1U
PLTGold® Human Platelet Lysate	PLTGOLD100GMP PLTGOLD100R
Dulbecco's Phosphate Buffered Saline (DPBS) no calcium, no magnesium	02-023-1
Recombinant Trypsin Solution or Recombinant Trypsin-EDTA Solution	03-078-1 03-079-1
Soybean Trypsin Inhibitor (SBTI) (50X) (optional)	03-048-1C

Reagent Storage and Notes

MSC NutriStem® XF Medium

- MSC NutriStem® XF Medium contains a basal medium and supplement mix. For specific applications, the addition of the supplement mix may be optional. Adding the supplement mix can further increase MSC proliferation and expansion rates.
- Store the basal medium at 4 °C, protected from light.
- Basal medium contains stable L-alanyl-L-glutamine. No additional L-glutamine is necessary.
- Store the supplement mix at -20 °C, protected from light.
- The frozen supplement mix should be thawed at room temperature or at 4 °C.
- If not using immediately, prepare single-use aliquots of the supplement mix from the stock solution and re-freeze at -20 °C. Avoid repeated freeze-thaw cycles (up to two times).

PLTGold® Human Platelet Lysate

- Thaw frozen PLTGold® 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 PLTGold® to repeated temperature changes or freeze | thaw cycles. Long-term storage of PLTGold® at 4 °C is not recommended.

NOTE:

PLTGold® Human Platelet Lysate does not require the addition of heparin or any anti-coagulant for use.

Culture Medium Preparation and Storage

To prepare 100 mL of complete MSC medium, aseptically add the combine the following components:	NutriStem® MSC Basal Medium	100 mL
	NutriStem® MSC Supplement Mix	0.6 mL
	PLTGold® Human Platelet Lysate	5.5 mL

To prepare 500 mL of complete MSC medium, aseptically add the combine the following components:	NutriStem® MSC Basal Medium	500 mL
	NutriStem® MSC Supplement Mix	3 mL
	PLTGold® Human Platelet Lysate	26 mL

Store the complete MSC culture medium at 4 °C, protected from light, for up to 2 weeks.

Protocol for Use

The following protocol outlines the routine culture procedure for expanding human MSC in MSC NutriStem® XF Medium and 5% PLTGold® Human Platelet Lysate. This protocol can be used as a guideline to establish optimal culture parameters for each unique MSC line or application.

Getting Started

- In general, cells do not require an extended adaptation phase when transitioning to MSC NutriStem® XF Medium supplemented with PLTGold® as the culture medium.
- Cells can be directly seeded in MSC NutriStem® XF Medium supplemented with PLTGold® upon a thaw.
- Cell seeding should be performed following the general guidelines for the specific cell type. Typically, human MSC are plated at approximately 2×10^3 to 5×10^3 cells per cm^2.
- Many MSC lines proliferate rapidly in this medium, and should be observed daily to determine the optimal time to passage.
- Prior to feeding or passaging, warm only the amount of medium that will be used that day. Discard any excess pre-warmed complete media at the end of each day.
- Perform a complete medium change every other day as needed between passages.

NOTE:

Observe cells daily and passage when the culture reaches 60% to 70% confluency.

Culture and Maintenance of MSC

For best results, MSC should be passaged when the cell confluency reaches 60% to 70%. Do not allow MSC to overgrow in culture (over 80% confluency). For many MSC lines, the cells proliferate rapidly in this medium, and may require passaging every 3 to 4 days. Perform a full medium exchange every other day while cells are being maintained in culture.

Passaging

1. Briefly warm a sufficient amount of complete culture medium 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^{2+} or Mg^{2+}) to wash the culture surface. Use approximately 2 mL of DPBS per 10 cm^2 culture surface area.
4. Gently rock the culture vessel to wash the cells, and aspirate the DPBS.
5. To detach the cells, add a sufficient volume of Recombinant Trypsin Solution to cover the cell culture surface, and incubate cells at room temperature or 37 °C for 4 to 5 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 3 minutes. Do not over-incubate the culture, as MSC can be sensitive to enzymatic stress.
7. Once the cells are detached from the culture surface, quench the trypsin by adding a volume of pre-warmed complete culture medium that is 4 times the volume of the Recombinant Trypsin Solution used. Alternatively, 1X Soybean Trypsin Inhibitor (SBTI) solution diluted in DPBS can be used to quench the trypsin.
8. Collect the cell suspension and transfer to a centrifuge tube. If needed, rinse the culture vessel with additional media to collect any remaining cells and transfer to the same tube.
9. Centrifuge at 200 × g for 5 minutes at room temperature.
10. Remove the supernatant and suspend the cell pellet in 5 mL of complete culture medium.
11. Perform a cell count and calculate viability, concentration (cells/mL), and total cell number.

NOTE:

Recombinant Trypsin-EDTA Solution can be used if cells are over-confluent or difficult to detach after a short incubation with Recombinant Trypsin Solution.

Passaging cont.

12. Plate the cells at a seeding density of 2×10^3 to 5×10^3 cells per cm^2, 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 pre-warmed complete medium can be added to reach the total volume required.
13. Incubate the cells overnight at 37 °C and 5% CO_2.
14. Observe cells daily to monitor cell health, proliferation, and confluence. Perform a complete medium change every other day or as needed between passages.

NOTES:

MSCs tend to proliferate rapidly in this culture medium. Avoid high seeding densities to allow the cells room to expand prior to passaging.

References

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