CAPTIVATE BIO

GUIDELINES FOR USE

# RESiVATE™ MSC Expansion Medium

## Overview

**Introducing RESiVATE™** – a new generation of xeno-free cell culture media designed for the rapid expansion of human mesenchymal stromal cells (MSCs). Captivate Bio's RESiVATE™ MSC Expansion Medium is a growth factor rich culture system that enables scientists the ability to customize culture conditions on the fly. Available with or without the supplement, the RESiVATE™ MSC Basal Medium is an optimized, baseline formulation that does not contain growth factors, cytokines, or unknown supplements, leaving you in full control of your experimental conditions. Used in combination with the basal medium, the hPL-based supplement provides essential proteins for consistent cell revival and growth.

The following components of the **RESiVATE™ MSC Expansion Medium** (Cat. No. CBMSKT) include the following:

| PRODUCT INFORMATION | CAT. NO. | SIZE | STORAGE | SHELF LIFE |
|---|---|---|---|---|
| RESiVATE™ MSC Basal Medium | CBMS01 | 500 mL | 2°C - 8° C | 12-months from date of manufacture |
| RESiVATE™ HPL Supplement | CBMS15 | 27 mL | - 20° C | Stable until expiry date on label |

None of the above components contain antibiotics. RESiVATE™ MSC Basal Medium is also sold separately for those researchers wanting to use their own supplements.

## Preparation of Complete RESiVATE™ MSC Expansion Medium

The following guideline outlines a routine culture procedure for initiating and expanding human, bone marrow-derived MSCs using Captivate Bio's RESiVATE™ MSC Expansion Medium. This guide can be used as a baseline to establish optimal culture conditions for your specific MSC line(s).

**Materials**

- **RESiVATE™ MSC Expansion Medium** (Captivate Bio, Catalog #CBMSKT)
- **DPBS** (Captivate Bio, Catalog #DPBS02 or DPBS12)
- **Human Fibronectin** (Optional: Sigma, Catalog #F1056-2MG)
- **Frozen vial of mesenchymal stem cells**
- **Culture vessels (T-25 or T-75)** (T-25 Captivate Bio, Catalog #707001) (T-75 Captivate Bio, Catalog #708001)
- **15 mL conical, centrifuge tubes** (Captivate Bio, Catalog #601051)
- **TrypLE® or Accutase®**

**Directions for Use**

Use sterile techniques to prepare the complete medium (RESiVATE™ MSC Basal Medium + RESiVATE™ HPL Supplement). The following guideline outlines preparation of the complete medium as provided. Please note that other volumes or concentrations may be further adjusted or optimized depending on your specific cell line.

1. Thaw frozen supplement in a (37°C) water bath or overnight at 2 - 8°C . Mix gently and thoroughly once thawed.
2. After thaw, add 27 mL of the supplement to 500 mL of the basal medium and mix thoroughly prior to use.
   **Tip:** If not used immediately, store the complete medium at 2 - 8°C, protect from light, for up to 2 weeks.
3. Do not exceed the shelf life of the individual components.

**Notes**

- In general, cells do not require an extended adaptation phase when transitioning to RESiVATE™ MSC Expansion Medium as the culture medium.
- Cells can be directly seeded in RESiVATE™ MSC Expansion Medium upon a thaw.
- Cell seeding should be performed following the general guidelines for your specific cell type. Typically, human MSCs are plated at approximately 4 x $10^3$ to 10 x $10^3$ cells per $cm^2$.
- Aliquot enough volume required for that day and allow time to come to room temperature prior to use. Discard any excess pre-warmed complete media at the end of each day.
- Avoid multiple freeze/thaw cycles where possible.

**Recovery and Plating Cryopreserved MSCs**

For best results, cryopreserved MSCs should be thawed rapidly to reduce cell exposure and cell death. It is important to prepare pre-coated fibronectin or vitronectin culture vessels (plates or flasks) for optimal attachment and growth. Additionally, prepare pre-warmed RESiVATE media and bring to room temperature prior to use. MSCs will attach within 24 hours and typically require 3-7 days of growth prior to the initial passage. MSCs may require an additional few days to reach confluence from initial thaw depending on factors such as cell line origin, cryopreservation method and storage conditions.

**Thawing Procedure**

1. Pre-warm a sufficient amount of complete RESiVATE™ MSC Expansion Medium at 37°C. A minimum of 10mL is needed for recovery of a single cryopreserved vial of MSCs in addition to the amount of media required for plating.
2. Prepare 15mL conical tube with 8mL of RESiVATE MSC Expansion Medium.
3. Ensure a pre-coated fibronectin plate is prepared by placing into incubator for at least 1 hour prior to use. (For best results, please reference the specific product instructions for the specific matrix being used).
4. Rapidly thaw the cryopreserved vial of MSCs by placing under warm water OR by placing in heat block until a small ice pellet remains.
5. Transfer vial to sterile biosafety cabinet hood for further processing.
6. Carefully pipet the cells into a 15mL conical tube prepared with 8mL of RESiVATE MSC Expansion Medium. Carefully wash the cryovial with fresh 1mL of RESiVATE MSC Expansion Medium and transfer to conical tube containing freshly thawed cells in RESiVATE MSC Expansion Medium.
7. Spin cells at 1200 rmp for 5 minutes.
8. Add half volume of RESiVATE MSC media to prepared pre-coated fibronectin plate.
9. Resuspend cells in 2mL of media and obtain cell count.
10. Adjust cell concentration such that the plating density is between 3,000-10,000 cells per $cm^2$.
11. Place plate with seeded cells into incubator overnight.
12. Change media the following day post thaw. Feed every other day or 3 times per week for up to 5-7 days before passaging for expansion.

**Passaging**

For best results, MSCs should be passaged when cell confluency reaches 60% to 80%. Do not allow MSCs to overgrow in culture. For many MSC lines, the cells grow rapidly in this medium, and passaging should be performed every 3 to 5 days. Perform a complete medium exchange every other day while cells are being maintained in culture.

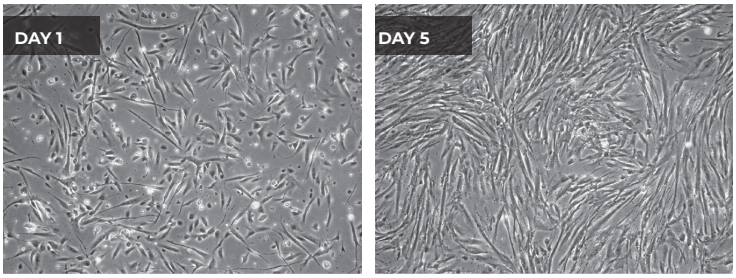


**Figure 1. Expansion of BM-MSCs cultured in RESiVATE MSC Expansion Medium.** MSCs were seeded at 8,000 cells/cm$^2$ on day 0 with image captured on day 1 and day 5 during initial passage. Typical "shoalike" morphology of MSCs was observed.

**Expansion of MSCs**

1. Pre-warm a sufficient amount of complete RESiVATE™ MSC Expansion 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 approximately 2 mL of DPBS (-/-) per 10 cm$^2$ to wash the culture surface.
4. Gently rock the culture vessel to wash the cells then aspirate the DPBS.
5. To detach the cells from the culture surface, add a sufficient volume of Accutase® (or TrypleE) to cover surface and incubate cells at room temperature or 37°C for 2 to 4 minutes. Tap the vessel periodically or gently titrate to expedite cell detachment.
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.
7. Once the cells are detached from the culture surface, collect the cell suspension and transfer to a centrifuge tube.
8. Wash cell plate with 1x volume of additional media to collect and transfer any remaining cells. Ensure that cell solution containing Accutase or trypsin is diluted at least 5x volume of the dissociation reagent using pre-warmed RESiVATE™ MSC Medium to prevent further activity of the dissociation reagent.
9. Centrifuge at 1200 x rpm for 5 minutes at room temperature.
10. Remove the supernatant and suspend the cell pellet in 2 mL of RESiVATE™ MSC Expansion Medium.
11. Perform a cell count and calculate viability, concentration (cells/mL), and total cell number.
12. Plate the cells at approximately 4 x 10$^3$ to 10 x 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 RESiVATE™ MSC Expansion Medium can be added to reach the total volume required.
13. Incubate the cells at 37°C.
14. Observe cells daily to monitor cell health, proliferation, and confluence. Perform a complete medium change every other day as needed between passages. If culturing over the weekend, ensure to change media the Friday before using double the volume.
    **Note:** Typically MSCs are cultured at 5% $O_2$ but can be cultured at normoxic levels if Tri-gas incubator is unavailable.