

# MesenPRO RS™ Medium

## Description

MesenPRO RS™ Medium is a reduced serum (2%) medium specifically formulated to support the growth of human mesenchymal stem cells (MSCs) in culture. MesenPRO RS™ Medium consistently improves MSC expansion compared with conventional serum-supplemented media while maintaining a comparable gene expression profile. Using MesenPRO RS™ Medium, MSCs can be expanded for multiple passages while maintaining their multipotential phenotype (i.e., differentiation into osteogenic, chondrogenic, and adipogenic lineages).

Product	Catalog No.	Amount	Storage	Shelf Life*
MesenPRO RS™ Medium**	12746-012**	1 kit		
Contains:				
MesenPRO RS™ Basal Medium	12747-010	500 mL	2°C to 8°C; Protect from light	24 months
MesenPRO RS™ Growth Supplement	12748-018	10 mL	-20°C to -5°C; Protect from light	24 months

\* Shelf Life duration is determined from Date of Manufacture.

\*\* MesenPRO RS™ Medium is sold as a complete kit, components are not sold separately.

## Intended Use

For *in vitro* diagnostic use.

## Important Information

- Thaw MesenPRO RS™ Growth Supplement at 2°C to 8°C, use immediately once thawed.
- Avoid repeated freeze/thaw cycles of MesenPRO RS™ Growth Supplement.

## Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Prepare Media

Supplement MesenPRO RS™ Basal Medium with MesenPRO RS™ Growth Supplement, and L-glutamine or GlutaMAX™-I prior to use.

1. Aseptically add 10 mL MesenPRO RS™ Growth Supplement to 500 mL MesenPRO RS™ Basal Medium.
2. Aseptically add 5 mL 200 mM L-glutamine or GlutaMAX™-I, 2 mM final concentration, to the medium before use.

Once supplemented, the complete MesenPRO RS™ Medium is stable for up to two weeks when stored at 2°C to 8°C protected from light. We do not recommend using beyond two weeks.

## Culture Conditions

**Media:** Complete MesenPRO RS™ Medium.

**Cells:** Human MSCs.

**Culture Type:** Adherent

**Culture Vessels:** T-flasks.

**Temperature Range:** 36°C to 38°C.

**Incubator Atmosphere:** Humidified atmosphere of 4–6% CO<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Recovery of Cryopreserved MSCs

**Note:** We recommend that you recover MSCs into conventional serum-supplemented medium (e.g., DMEM with 10% MSC Qualified FBS) from thaw, then subculture cells directly into complete MesenPRO RS™ Medium.

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Pipet the entire contents of the cryovial into a sterile 50-mL conical tube.

3. Carefully, by dropwise addition (2–3 drops per 10 seconds), add 5–7 mL of prewarmed complete conventional serum supplemented media while gently swirling the tube.
4. Add a further 5 mL prewarmed complete conventional serum-supplemented media while gently swirling the tube.
5. Transfer all the contents of the conical tube into an appropriate tissue culture flask.
6. Incubate at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air.
7. Exchange spent medium with fresh prewarmed complete conventional serum-supplemented media 24 hours post-thaw.  
**Note:** For recovery of MSCs, it is recommended to seed cells at  $\geq 7 \times 10^3$  cells/cm<sup>2</sup> for the initial recovery passage.

## Subculture MSC

MesenPRO RS™ has been developed for the culturing of human mesenchymal stem cells that have been initiated using standard adherent isolation and growth conditions (e.g., DMEM + 10% FBS) and has not been tested for the initial expansion of MSCs directly from primary tissue sources. MesenPRO RS™ has been developed for culturing MSCs at greater than clonal densities (see step 9).

It is recommended to subculture human mesenchymal stem cells directly into MesenPRO RS™ complete medium. It is critical that cell confluency be 60–80%, cell viability be at least 90%, and the growth rate be in mid-logarithmic phase prior to sub culturing.

**Note:** The following procedures apply to adherent cultures in a T-75 culture flask (75 cm<sup>2</sup>). Volumes should be adjusted accordingly for desired vessel size.

1. Observe culture flask on inverted microscope and confirm that the cells are ready to be subcultured (60–80% confluent).
2. Aspirate medium and floating cells from a confluent monolayer and discard.
3. Wash cells with 5–10 mL prewarmed Dulbecco's Phosphate Buffered Saline (DPBS), without calcium, magnesium, or phenol red.
4. Remove DPBS and add 5–7 mL of prewarmed TrypLE™ Express to the culture flask. Incubate at 37°C until cells have fully detached (approximately 3–5 minutes).
5. Observe cell monolayer using an inverted microscope to ensure complete detachment from the surface of the flask.
6. Stop cell dissociation by adding 10 mL of prewarmed complete MesenPRO RS™ Medium to flask. Gently pipet up and down to disperse cells into a single-cell suspension.

7. Transfer cell suspension into a sterile conical tube. Wash flask with an additional 5 mL prewarmed complete MesenPRO RS™ Medium and combine into the conical tube.
8. Centrifuge cell suspension at 100 × g for 5–10 minutes.
9. Aspirate supernatant and resuspend the pellet in an appropriate volume of prewarmed complete MesenPRO RS™ Medium.
10. Determine total viable cell density using a Countess® Automated Cell Counter (alternative automated or manual methods may be used).
11. Inoculate flask at 3–5 × 10<sup>3</sup> viable cells/cm<sup>2</sup> and return to incubator.












**Note:** For optimal performance and cell growth, cultures should be re-fed every 3–4 days with fresh complete medium.

## Related Products

Product	Catalog No.
GlutaMAX™-I, 200mM (100X), Liquid	35050
L-Glutamine-200mM (100X), Liquid	25030
Dulbecco's Phosphate Buffered Saline, without calcium, magnesium or phenol red	14190
DMEM/F-12, GlutaMAX™	10565
DMEM, low glucose, pyruvate, no glutamine, no phenol red	11054
Fetal Bovine Serum, MSC-Qualified (USDA Approved)	12662
TrypLE™ Express (1X), no Phenol Red	12604
StemPro® Accutase®	A11105
StemPro® MSC SFM	A10332-01
StemPro® MSC SFM XenoFree	A10675-01
Gibco® Mouse (C57BL/6) Mesenchymal Stem Cells	S1502-100
StemPro® Rat Alk Phos Expressing Mesenchymal Stem Cells	R7789
Gibco® Rat (SD) Mesenchymal Stem Cells	S1601-100
StemPro® Adipogenesis Differentiation Kit	A10070
StemPro® Chondrogenesis Differentiation Kit	A10071
StemPro® Osteogenesis Differentiation Kit	A10072
StemPro® Human Adipose-Derived Stem Cell Kit	R7788
Gentamicin Reagent Solution (50 mg/mL), Liquid	15750
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

## Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

			
Caution, consult accompanying documents	In vitro diagnostic medical device	Sterilized using aseptic processing techniques	Protect from light
			
Use By:	Catalog number	Manufacturer	Batch Code
			
European Community	Consult instructions for use	Temperature Limitation	

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support)  
For further assistance, email [techsupport@lifetech.com](mailto:techsupport@lifetech.com)

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