

MSC Expansion Medium
for Bone Marrow
Derived MSCs Kit Culture

Product Information

Product Name	Size	Final Concentration in Supplemented Media	Storage Temperature
MSC Expansion Medium	485mL	N/A	2 - 8°C
RH IGF-1	0.5mL	15ng/mL	-20°C
RH FGF-B	0.5mL	125pg/mL	-20°C
L-ALA-L-GIN	6mL	2.4mM	-20°C
FBS	35mL	7%	-20°C
G-A (Optional Antimicrobial containing Gentamicin and Amphotericin B)	0.5mL	Gentamicin 30µg/mL Amphotericin B 15ng/mL	-20°C

Preparation of reagents

Media Preparation

The basal media contains no growth factors, antimicrobials, or phenol red. To support cell proliferation, the supplements must be added to the basal medium.

Basic Aseptic Technique

Axol's MSC Expansion Medium for Bone Marrow Derived MSCs Kit should only be used in an aseptic environment, a Class II biological safety cabinet with front access and filtered laminar airflow, or an equivalent device. Always wear gloves and eye protection when working with these materials. Wipe or spray all bottles and vials with 70% ethanol or isopropanol, especially around the area of the cap, before placing them in the biological safety cabinet.

Allow these surfaces to dry completely before opening the bottle or vials. Always transfer medium and supplements with disposable sterile pipettes. Do not mouth pipette. Take up the volume needed into the pipette, being careful not to touch the sterile tip to the rim of the container or any other surface. Close the container and open the container into which the transfer is being made, again being careful not to touch any surfaces with the sterile tip.

Transfer the material and close the container. Wash your hands before and after working with cell cultures. Do not block airflow in a laminar flow hood as this may compromise sterility. Ensure that biological safety cabinets are certified routinely and that the HEPA filters are replaced regularly.

Adding Supplements

When supplementing basal medium, Axol recommends the use of a suitable pipette to remove the exact volume of growth factor or supplement from each supplement. The supplements are manufactured with a slight overfill to ensure that the full labelled volume is present. The cells will still perform within guaranteed culture specifications if the entire volume of growth factor or supplement is directly added to the basal medium. However, for consistent performance results, please add the exact volume of growth factor or supplements as per the table provided in these instructions.

All procedures should be executed using aseptic technique (see section on basic aseptic technique). The supplements are sufficient to supplement one 485 mL bottle of Basal Medium. The supplements should be thawed and mixed immediately prior to supplementation. Mix supplemented medium by gently pipetting up and down with a large volume pipette (25 or 50 mL) or gently invert the tightly closed 500 mL bottle. Do not shake or froth the medium. The supplemented medium may be stored at 2 to 8°C for up to two weeks.

Pre-warming Medium

If using less than 100 mL of complete medium, Axol recommends warming only the volume needed in a sterile conical tube. Repeated warming of the entire bottle over extended periods will cause degradation and reduce the shelf life of the medium. When warming the entire bottle of medium, Axol recommends using a water bath sleeve to help protect the medium from contaminants in a 37°C water bath. Medium will warm to 37°C in 10 to 30 minutes, depending on the volume. Do not leave medium in water bath for extended periods of time.

Recommended Feeding Guidelines:

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area: Every other day, remove medium and feed with 5 mL of fresh supplemented medium.

Most cultures that are 50% confluent will be ready for passage the following day and should be fed with 7 to 8 mL of supplemented medium.

Do not use more than 10 mL of supplemented medium per 25 cm² of culture surface to ensure that the media is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended result in a range of depths between 2mm and 5mm which is comparable with general recommendations with 10mL being the maximum depth allowable (5mm).

G-A (Gentamicin and Amphotericin B):

A vial of GA is provided with the Kit for your convenience as an optional supplement. The use of GA is recommended to inhibit potential fungal or bacterial contamination of eukaryotic cell cultures. GA is best stored at -20°C; it should only be thawed once and stored at 4°C for a maximum of two weeks after thaw. Addition of 0.5 mL of GA to 500 mL of media will provide an effective concentration to inhibit bacterial and fungal cell division.

Notes

Got any questions? Need help with the protocol?
Contact Axol Technical Support at
support@axolbio.com
Or
call +44 (0) 1223 751051