

AllPhase Supplement 20X

REF **AGA1000-S****-20°C****Protect from light**Phenol Red **No**

❖ Supplement Specifications

Brand: Duogenic StemCells corporation**Form:** Liquid**Cells:** BM-MSC, ADSC, UC-MSC**Storage:** -20°C**Shelf Life:** 12 months

❖ Preparation Complete AllPhase- MSC medium

Thaw AllPhase Supplement 20X at 4°C for 20-24 hours before use. Avoid freeze/thaw cycles. Aseptically add 50 ml of AllPhase Supplement 20X in 1 Liter of IMDM (Gibco or Simply). Store complete AllPhase-MSC medium at 2-8°C protected from light. We recommend the use of the complete medium within a period of 3 months.

❖ Coating

No coating. We recommend the use of Corning® CellBIND® surface vessels as it provides a better attachment for WJ-MSC. However, the same expansion rate was observed by culturing WJ-MSC, ADSC, BM-MSC on tissue culture treated vessels. See **table 3.1 and 3.2** the tested brands.

❖ Product description

AllPhase Supplement 20X is an optimized serum-free and xeno-free supplement recommended for the growth and expansion of *ex vivo* human mesenchymal stem cells (hMSCs), including bone marrow mesenchymal stem cell (BM-MSC), adipose-derived stem cell (ADSC) and umbilical cord-derived mesenchymal stem cell (UC-MSC).

Human MSCs cultured with AllPhase Supplement 20X keep an excellent expansion rate after several passages and maintain their self-renewal and multi-lineage differentiation potential in coating-free condition.

❖ Culturing hMSC with AllPhase Supplement 20X

Recovery

1. Pre-warm 5 ml of AllPhase-MSC complete medium in a conical tube.
2. Thaw the vial at 37°C in a water bath for 1-1m30, a small amount of ice should remain.
3. Slowly transfer the cells into the AllPhase-MSC complete medium.
4. Centrifugate the cells at 200g for 3 min at room temperature.
5. Remove the supernatant and resuspend the pellet in 1ml of AllPhase-MSC complete medium.
6. Count the viable cells by using the Trypan Blue assay.
7. Seed the desired number of cells. The recommended seeding density is shown in **Table 1**.
8. Incubate at 37°C in a 5% CO₂ incubator.
9. Replace the medium with fresh complete AllPhase-MSC medium every 2-3 days.

❖ Additional products

- Iscove's Modified Dubelcco's Medium (IMDM) from Gibco (cat. number 12440046)
- Corning® CellBIND® surface vessels
- TrypLE enzyme (cat. 12605-028 Gibco) or a mix of 0.25% Trypsin (cat. 27250-018 ThermoFisher) + 0.53 mM EDTA (cat. 0105-1KG AMRESCO)

Table 1. Recommended seeding density limits

Culture ware	Surface (mm ²)	Seeding densities (cells/well)	
		Lower	Higher
6-well	962	0.5-1.0 x10 ⁴	4.0-6.0 x10 ⁴
12-well	401	0.2-0.4 x10 ⁴	1.6-2.4 x10 ⁴
T-25 flask	2,500	1.25-2.5x10 ⁴	10.0-15.0 x10 ⁴
T-75 flask	7,500	3.75-7.5x10 ⁴	30.0-45.0 x10 ⁴

Seeding density	Passage frequency (days)	Max passages	Cell types
4.0x10 ⁴	3	9-13	ADSC, WJ-MSC, BM-MSC
1.0x10 ⁴	5-6	9-11	WJ-MSC
0.5x10 ⁴	7-9	6	BM-MSC

Table 2. Recommended amount of TrypLE or Trypsin+EDTA for a confluence of 80%.

Culture ware	TrypLE (ml)	0.25%Trypsin+ 0.53 mM EDTA (ml)
6-well	1.0	1.0
12-well	0.5	0.5
T-25 flask	2.0	2.0
T-75 flask	4.0	4.0

No.18, Ln.10, Taiping 21st St., Taiping Dist., Taichung City 41161, Taiwan (R.O.C.)

(+886) 04-22766193
www.duogenic.com

❖ Culturing hMSC with AllPhase Supplement 20X

Subculturing MSC

We recommend to subculture the hMSCs when the cells confluence reach up to 80%. The hMSCs can be seeded at a concentration of 4000-6000 cells/cm² (see **Table 1**).

Protocol

1. Pre-warm AllPhase-MSC complete medium and 0.25% Trypsin+ 0.53 mM EDTA or TrypLE.
2. Remove the culture medium and add the appropriate amount of TrypLE or Trypsin+EDTA (see **Table 2**). The volume should properly cover the entire cells surface.
3. Incubate 1 to 2 minutes at 37°C and verify the detachment. Tap the flask gently if necessary.
4. Add the same volume of AllPhase-MSC complete medium as Trypsin+EDTA or TrypLE.
5. Transfer the cells into a sterile conical tube and centrifugate at 200g for 3 minutes at room temperature.
6. Discard the supernatant and resuspend the cells pellet in 1–2 ml of AllPhase-MSC complete medium.
7. Count the viable cells by using the Trypan Blue assay.
8. Seed the desired number of cells. The recommended seeding density is shown in **Table 1**.
9. Incubate at 37°C in a 5% CO₂ incubator.
10. Replace the medium with fresh complete AllPhase-MSC medium every 2-3 days.

Cryopreservation of hMSCs

Prepare the cryopreservation solution on the same day.

Cryopreservation solution preparation

AllPhase-MSC complete medium + 8% Dimethyl Sulfoxide (DMSO). Keep on ice.

Protocol

1. Resuspend the cells into the cryopreservation solution (We recommend 0.5-1x10⁶ cells/ml 1ml/vial).
2. Immediately place the cells into an automated or manual freezing rate-controlled apparatus (decrease 1°C/minute). Or place the vials into freezing containers and place at -80 °C overnight.
3. After 24 hours, transfer the vials into liquid nitrogen.








Table 3.1 Culture ware tested brand with similar hMSCs expansion rate.

Brand	Cat.
Corning® CellBIND®	3335
TPP® tissue culture plates	92006
SPL® Cell Culture Plate	30006
CELLSTAR®	657160
Corning® Costar®	3516
Nunc™	140675
Falcon®	353046
Nunc™ EasYFlask™ Cell Culture Flasks	159910; 156499; 156367
Falcon® Cell Culture Multi-Flask	353143
Corning® Primaria™ Cell Culture Flasks	353810

Table 3.2 Culture ware with similar hMSCs expansion rate based on WJ-MSC, ADSC and BM-MSC.

Cat.	ADSC	WJ-MSC	BM-MSC
3335	•	•	•
92006	•		
30006	•		
657160	•		
3516	•		
140675	•		
353046	•	•	•
159910; 156499; 156367	•	•	•
353143		•	
353810		•	

❖ Explanation of symbols of warning

	The manufacturer's catalogue number
	The manufacturer's batch code
	Specifies the expiration date
	Indicates the temperature limits to which expose the product
	The product must be protected from light
	Indicates the date when the product was manufactured
	The user needs to consult the instruction of use

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