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Technical Datasheet

HiMesoXL™ Mesenchymal Stem Cell Expansion Medium

Product Code: AL512

Intended Use:

Designed for in vitro cultivation and expansion of Human Wharton's Jelly Mesenchymal stem cells, Human adipose derived mesenchymal stem cells and Human dental pulp stem cells.

Principle & Interpretation:

HiMesoXL™ Mesenchymal Stem Cell Expansion Medium is designed for in vitro cultivation and expansion of Human Wharton's Jelly Mesenchymal Stem Cells (HWJ-MSC), Human Adipose Derived Mesenchymal Stem Cells (HAD-MSC) and Human Dental Pulp Stem Cells (HDP-SC) while maintaining them in an undifferentiated state. This medium is a proprietary formulation containing inorganic and organic salts, amino acids, vitamins, growth factors, nutrients and sodium bicarbonate. The product does not contain antibiotics and antimycotics.

Composition:

Proprietary

Type of specimen:

1. Umbilical cord
2. Lipoaspirate
3. Fat tissue
4. Dental pulp

Specimen collection & Handling:

1. Umbilical cord

- Human umbilical cord is collected by obstetrician/ gynecologist at the hospital while giving birth.
- After the baby is born, double clamp the umbilical cord.
- Apply the first clamp near placenta.
- Apply the first clamp to the cord on the baby side.
- Cut the cord &drain the blood into the sterile bottle.
- Collect the cord in the sterile collection bottle & store at 2-8ºC.
- Ship it to the processing laboratory within 24-48 hours.

2. Lipoaspirate

- Lipoaspirate sample is collected from male & female adult patients undergoing voluntary liposuction by a cosmetic surgeon.
- Collect the sample in sterile collection bottle & store at 2-8ºC.
- Ship it to the processing laboratory within 24-48 hours.

3. Fat tissue

- Fat tissue is collected by an authorized medical practitioner.
- Cut the fat tissue into small pieces.
- Collect the tissue in sterile collection bottle & store at 2-8ºC.
- Ship it to the processing laboratory within 24 -48 hours.

4. Dental pulp

- Human teeth is collected by an authorized dental surgeon/dentist.
- Oral surgeons remove any loose soft tissue & place extracted teeth into sterile chilled vials containing 20ml phosphate buffered saline.
- Collect the teeth in sterile collection bottle & store at 2-8ºC.
- Ship it to the processing laboratory within 24 - 48 hours.

Warning and Precautions:

In Vitro Diagnostic Use only. This Product Should be used by trained healthcare professionals/ lab technicians only. Not to be used for self-testing. Read the label before opening the container. Wear protective gloves/ protective clothing/ eye protection/ face protection. Follow proper aseptic techniques while handling specimens and cultures. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety datasheets.

Directions:

Disinfect the external surface of the bottles of AL512 and FBS by spraying with isopropyl alcohol before placing in a biosafety hood.

1. Aseptically add FBS in AL512 in an amount equal to 10% of the total volume of complete medium. e.g. For 100ml medium, add 11ml FBS. For 500ml medium, add 55ml FBS.
2. If desired, 1.1ml of antibiotic-antimycotic solution (A002) can be added to 100ml of complete medium OR 5.5ml of antibiotic-antimycotic solution (A002) can be added to 500ml of complete medium.
3. Tightly cap the bottle and swirl gently to ensure proper mixing.
   Note: Do not mix vigorously. Doing so will cause formation of foam.
4. Store the complete medium at 2 - 8oC until use.

Products Required But Not Supplied:

1. Media Supplements	Code
Mesenchymal Stem Cell Tested Fetal Bovine Serum (FBS)	RM10832, RM10845 RM10846, RM10938
Antibiotic-Antimycotic Solution 100X [or] Gentamicin-Amphotericin B solution 1000X	A002 A031
2.Reagents for Sub-culture	**Code**
Dulbecco's Phosphate Buffered Saline (DPBS)	TL1006
Trypsin/EDTA Solution 1X	TCL007
Trypan Blue 0.5% solution	TCL005
3. Stem Cell Freezing Medium	**Code**
CryoXL™ Stem Cell Freezing Medium	TCL107

Limitations:

Not Applicable

Quality control:

Appearance
Orangish red colored clear solution

pH
7.00 - 7.60

Osmolality in mOsm/Kg H_2O
300.00 - 340.00

Sterility
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response
The medium is tested for optimal cell growth and proliferation of mesenchymal stem cells.

Quality Assurance:

- For in vitro diagnostic use. The medium is not intended for therapeutic use.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- Manufactured under ISO 13485 and ISO 9001 QMS.
- Manufactured under controlled environments and processes in accordance with ISO 13408 – Aseptic Processing of Health Care Products.

Disposal:

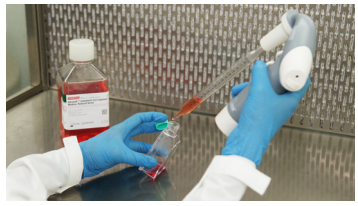
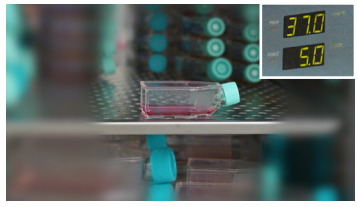
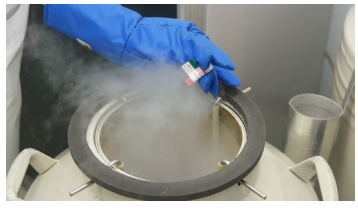
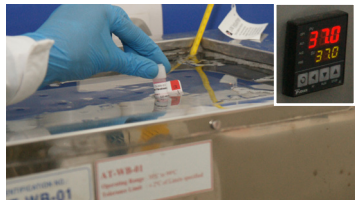
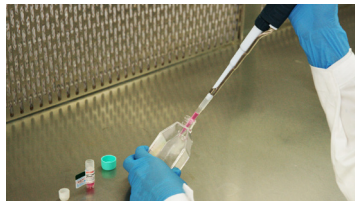
User must ensure safe disposal by autoclaving and /or incineration of used or unusable preparations of this product. Follow established laboratory procedures for disposing infectious materials. The materials that come into contact with clinical samples must be decontaminated and disposed of in accordance with current laboratory techniques [1, 2].

Storage and shelf life:

Store at 2-8ºC away from bright light. Note: Once complete medium has been formulated, store it at 2-8°C until use. Avoid extended exposure of complete medium to room temperature or higher temperatures. Complete medium should be equilibrated at room temperature before adding to cells. Freezing of the medium is not recommended.

Table 1: Protocol for thawing

- Cryopreserved cells are supplied in liquid nitrogen dry vapour shipper (-150ºC to -130ºC).
- Upon receipt, immediately transfer the vial to the vapor phase of liquid nitrogen tank.
- Store it in the tank until further use. Cells must be processed at least in a BSL II hood.

		Key Points to Remember	Time Required (approx.)
1. Preparation of Culture Vessel			
a. Add 5ml of complete medium to a T-25 flask.		**Preparation of complete medium AL512 (500 ml) + FBS (55ml) + A002 (5.5 ml).**	60 secs
b. Place the flask at 37°C to equilibrate the medium.			30 mins
2. Thawing Procedure		**Make sure water bath is set at 37ºC before starting the thawing procedure**	
a. Remove cryovial from the liquid nitrogen tank/ shipper wearing appropriate protective gear.		**Thawing should be AS FAST AS POSSIBLE to minimize cell damage.**	
b. Immediately thaw the vial partially by holding in a water bath at 37°C.		**DO NOT hold the vial in water bath for more than 90-120 secs.** **AVOID getting water upto the cap of the vial.**	90-120 secs
c. Disinfect the vial by swabbing thoroughly with 70% isopropyl alcohol.			10 secs
d. Add the cell suspension drop by drop to the T-25 flask containing the pre-warmed complete medium. Keep swirling the flask while adding the cell suspension.		**Dropwise addition is required to prevent the cells from stress induced by exothermic reaction.**	30-60 secs

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- Upon receipt, immediately transfer the vial to the vapor phase of liquid nitrogen tank.
- Store it in the tank until further use. Cells must be processed at least in a BSL II hood.

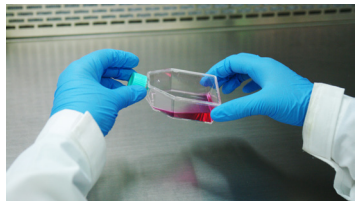
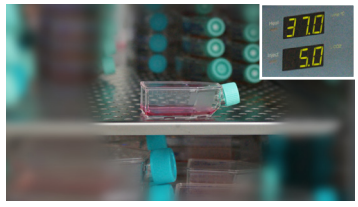
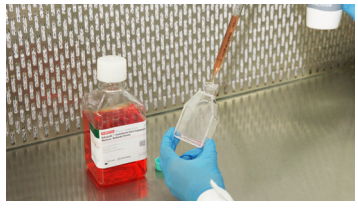
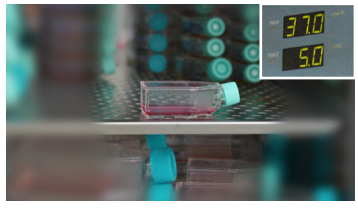
		Key Points to Remember	Time Required (approx.)
e. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium.			10 secs
3. Incubation			
a. Incubate the cells at 37°C and 5% CO_2.		**Check for cell attachment in 2-3 hrs**	2-3 hrs
b. If more than 70-80% cells are attached, replace the medium with fresh medium.		**Medium change after 2-3 hours is mandatory to remove traces of DMSO** **If cells have not attached, centrifuge the cell suspension at 1000 rpm for 7-8 mins and resuspend in fresh medium.**	60-120 secs 7-8 min
c. Incubate the cells at 37°C and 5% CO_2.			3-5 days
YOUR CELLS ARE READY TO SUB-CULTURE			
4. Maintenance			
a. Monitor the cells every day. b. Change the medium every alternate day. c.Sub-culture, once cells reach 70 - 80% confluence.		**Use the recommended freezing medium for cryopreservation of cells.** **DO NOT allow cells to reach 100% confluency before sub culture or cryopreservation.** **In case of reduced serum or serum free media, use trypsin inhibitor solution (TCL068) for neutralisation of Trypsin during subculture.** **Usage of just medium for neutralisation will result in inefficient neutralisation and will stress the cells resulting in reduced viability and cell death.**	

Table 2 : Sub-culture

- **HWJ-MSC/HAD-MSC/HDP-SC** can be sub-cultured at a seeding density of 5000-10,000 cells/cm^2.
- Sub-culturing ratios can vary from 1:2 - 1:5.
- A confluent T-25 flask of HWJ-MSC/HAD-MSC/HDP-SC yields 1.0 x 10^6 cells.

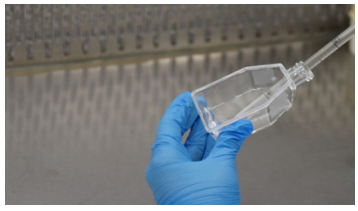
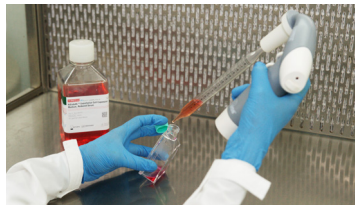
		Key Points to Remember	Time Required (approx.)
a. Aspirate entire medium and discard. DO NOT disturb the monolayer.		Keep the following ready : 1.New T25 surface treated flask for further subculture. 2.Required media and reagent should be at RT. 3.One 50ml centrifuge tube.	60 secs
b. Wash the cells with 2-3 ml DPBS to remove residual medium. c. Aspirate off the DPBS and discard.		**Prior to use, make sure that Trypsin-EDTA solution TCL137 is Prewarmed in a waterbath at 370 C for 30 mins before use .**	60 secs
d. Add 0.5 ml pre-warmed Trypsin-EDTA solution.		**Gently rock the flask to ensure complete coverage of the Trypsin-EDTA solution over the cells.**	
e. Incubate the flask in the incubator at 37°C for 30-60 secs.		**Exposing the cells to Trypsin-EDTA for longer time leads to loss of cell viability.**	30-60 secs
f. Microscopically monitor the flask. g. When the cells start rounding up, gently tap the flask to ensure complete detachment of cells.			15 secs
h. To neutralize action of trypsin add 3 ml of complete medium (AL512 + 10% FBS). i. Pipette gently to get a homogenous mixture of cells. j. Centrifuge the cell suspension at 600 rpm for 3 mins. Discard supernatant and resuspend pellet in fresh 3 ml of complete medium by pipetting.		**Vigorous pipetting will stress the cells.**	60 secs

Table 2 : Sub-culture

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- Sub-culturing ratios can vary from 1:2 - 1:5.
- A confluent T-25 flask of HWJ-MSC/HAD-MSC/HDP-SC yields 1.0 x 10^6 cells.

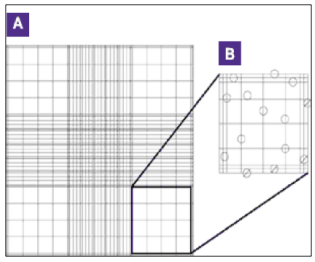
		Key Points to Remember	Time Required (approx.)
k. Count cells using hemocytometer. l. Seed at recommended seeding density in a new flask containing fresh complete medium. Refer to Table 3		**DO NOT refrigerate cells after splitting Seed immediately.**	10-15 mins
m. Incubate in a humidified incubator at 37ºC and 5% CO_2.			48 hrs
Maintenance			
a. Monitor the cells every day. b. Change the medium every alternate day. c. Sub-culture once cells reach 70 - 80% confluence.			

Table 3 : Seeding Density

Flask	Recommended Seeding Density	No. of Cells Per Flask	Volume of Medium (ml)
T-25	5000 cells/cm^2	0.125 x 10^6	5 - 7
	10,000 cells/cm^2	0.25 x 10^6	5 - 7

These are recommended seeding densities from literature and our studies. Higher seeding densities do not cause any harm to the cells and reduce the required population doublings per passage. Lower seeding densities may cause cells to lose viability, detach during culture and in general take more population doublings to reach confluence.

Related products:

Product Name	Code	Packing
HiFi™ Wharton's Jelly Derived Mesenchymal Stem cells (HWJ-MSC)	CL001-0.5 CL001-T25	0.5 million cells/vial 1 x T25cm^2 flask
HiFi™ Adipose Derived Mesenchymal Stem cells (HAD-MSC)	CL007-0.5 CL007-T25	0.5 million cells/vial 1 x T25cm^2 flask
HiFi™ Human Dental Pulp Stem cells (H-DPSC)	CL008-0.5 CL008-T25	0.5 million cells/vial 1 x T25cm^2 flask
HiMesoXL™ Mesenchymal Stem Cell Expansion Medium, Reduced serum	AL519-500ML	500ml
HiAdipoXL™ Adipocyte Differentiation Medium	AL521-100ML	100ml
HiOsteoXL™ Osteocyte Differentiation Medium	AL522-100ML	100ml
HiChondroXL™ Chondrocyte Differentiation Medium	AL523-100ML	100ml
EZXpand™ Mesenchymal Stem Cell Culture Kit (Adipose-derived)	CCK024-0.5 CCK024-T25	0.5 million cells/vial 1 x T25cm^2 flask
EZXpand™ Mesenchymal Stem Cell Culture Kit (Wharton's Jelly derived)	CCK025-0.5 CCK025-T25	0.5 million cells/vial 1 x T25cm^2 flask
CryoXL™ Stem Cell Freezing Medium	TCL107-50ML	50ml
Accutase™	TCL075-100ML TCL075-500ML	100ml 500ml
Trypsin-EDTA Solution 1X	TCL007-100ML TCL007-500ML	5 x 100ml 6 x 500ml
Dulbecco's Phosphate Buffered Saline	TL1006-100ML TL1006-500ML	5 x 100ml 6 x 500ml
Antibiotic Antimycotic solution 100X, Liquid	A002-20ML A002-50ML A002-100ML	5 x 20ml 5 x 50ml 5 x 100ml
Gentamicin Solution w/ 50mg/ml Gentamicin in sterile tissue culture grade water	A005-20ML A005-100ML	20ml 1 x 100ml
Gentamicin Amphotericin B Solution 1000X w/ 30mg/ml Gentamicin and 25µg/ml Amphotericin B in sterile cell culture grade water	A031-20ML	20ml 5 x 20ml
Sure Mesenchymal Stem Cell Tested Sera	RM10832-500ML RM10845-500ML RM10846-500ML RM10938-500ML	500ml 500ml 500ml 500ml

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
2. Jorgensen, J. H., Pfaller, M.A., Carroll, K.K.C., Funke, G. Landry, M.L., Richter, S and Warnock., D.W. (2015), Manual of Clinical Microbiology, 11th Edition. Vol. 1

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www.himedialabs.com

Product Label Symbols

REF	Catalogue number for product identification.
LOT	Batch/lot number for the batch identification.
	Date of expiry/ shelf life.
	Recommended storage temperature.
STERILE A	Manufactured using accepted aseptic techniques.
CE	Product meets the applicable EC directives requirements.
IVD	Product designed for use as an in vitro diagnostic medical device.
	Instructions for use.
BIO	Contains biological material of animal origin.
2	Single use. Not intended to be reprocessed and/ or used on another patient.