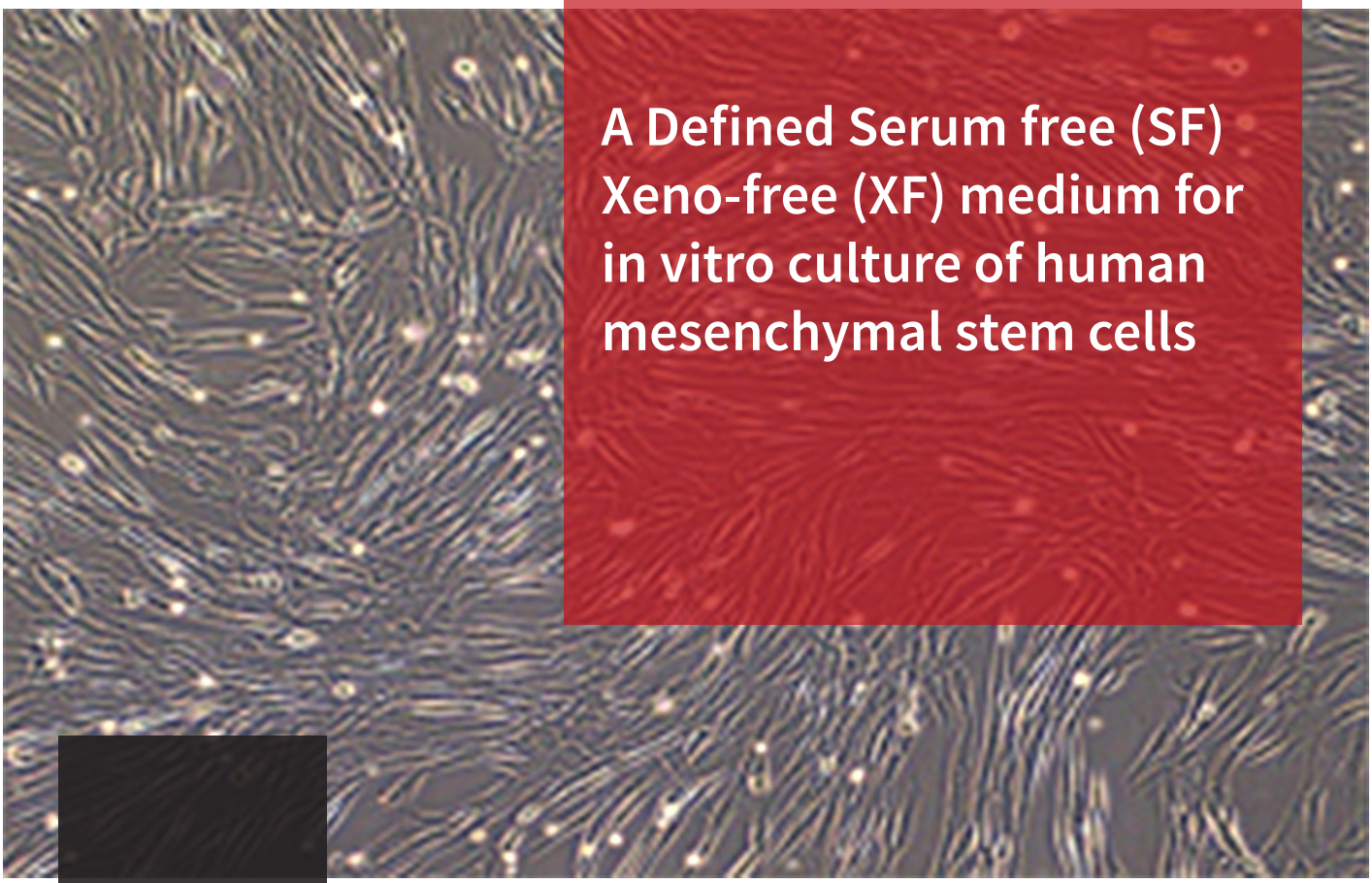
STEMin1™



A Defined Serum free (SF) Xeno-free (XF) medium for in vitro culture of human mesenchymal stem cells

HIMEDIA®
For Life is Precious

HIMEDIA®
Cell Culture
Enabling Breakthroughs

Product Portfolio

Expansion

STEMin1™ Defined, Serum-free, Xeno-free medium for expansion of human stem cells	AL520-500ML

Attachment

STEMin1™ Attachment Solution	TCL206-10ML TCL206-5x10ML

Dissociation

STEMin1™ Dissociation Solution	TCL208-100ML
STEMin1™ Neutralizer	TCL209-100ML

Cryopreservation

FREEZin1™ Universal freezing medium w/ DMSO w/o Antibiotics, Antimycotics and Phenol red	TCL098-50ML

Differentiation Media

HiAdipoXL™ Adipocyte Differentiation Medium	AL521-100ML
HiOsteoXL™ Osteocyte Differentiation Medium	AL522-100ML
HiChondroXL™ Chondrocyte Differentiation Medium	AL523-100ML
HiAdipoXL™ Adipocyte Differentiation Supplement	TCL167-1NO
HiOsteoXL™ Osteocyte Differentiation Supplement	TCL168-1NO
HiChondroXL™ Chondrocyte Differentiation Supplement	TCL169-1NO
HiAdipoXL™ Adipogenic Differentiation Medium for 3T3-L1 cells	AL537-100ML

Staining Kits

EZstain™ Adipocyte Staining Kit	CCK013-1KT
EZstain™ Chondrocyte Staining Kit	CCK029-1KT
EZstain™ Osteocyte Staining Kit	CCK030-1KT
EZstain™ Alkaline Phosphatase Staining Kit	CCK077-1KT

Cytokines & Growth Factors

Others

Antibiotic Antimycotic Solution 100X Liquid	A002-20ML A002-50ML A002-100ML
Gentamicin-Amphotericin B Solution 1000X	A031-20ML
Dulbecco's Phosphate Buffered Saline 1X	TL1006-100ML TL1006-500ML TL1006-1000ML

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STEMin1™

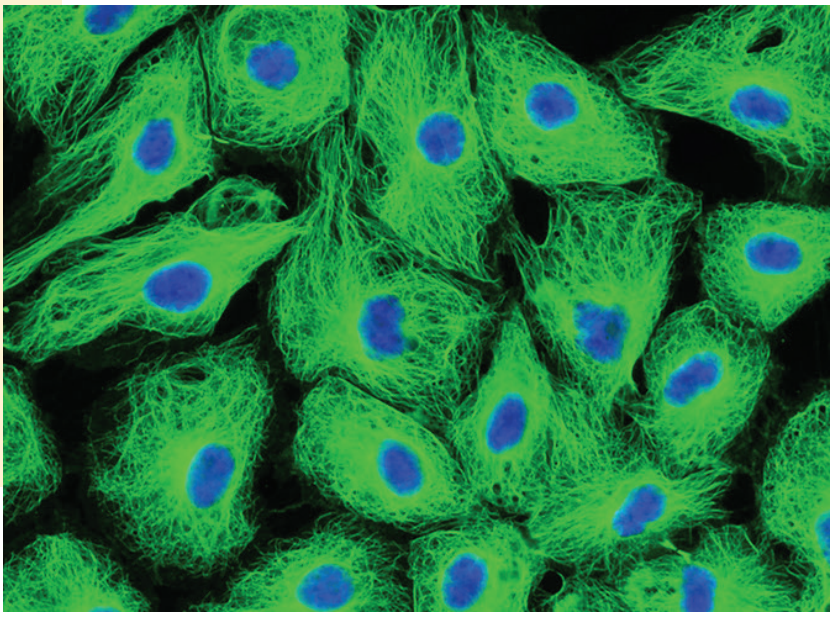
A Defined, Serum free (SF), Xeno-free (XF) medium for *in vitro* culture of human mesenchymal stem cells

Stem cells present multiple potential applications in regenerative medicine and are the subject of intense research. They constitute a very promising part of regenerative medicine and have many potential clinical applications. Harnessing their ability to replicate and differentiate into many cell types can enable successful treatment of diseases that were incurable until now.

Therapeutic applications of MSCs requires a wide range of in vitro conditions that mimic their in vivo human conditions demands in order to reach appropriate cell numbers that can achieve therapeutic outcomes. Thus, identification of optimal culture conditions is a prerequisite for MSC clinical applications. Addition of fetal bovine serum (FBS) to culture media provides the cells with vital nutrients, attachment factors, and growth factors. However, utilization of animal derived products bears critical limitations and safety concerns, such as animal derived (Xeno) antigens and infectious agents present in FBS that can lead to a risk of infusion reactions and transmission of zoonotic diseases to the recipient of MSCs therapy. STEMin1™ is a serumfree xenofree alternative designed for *in vitro* culture of human mesenchymal stem cells.

As an advanced therapy medicinal product, STEMin1™ meets special requirements includsing product specifications requiring a production process compatible with good manufacturing practice (GMP).

- Serum free and Xeno-free medium
- Manufactured in GMP, ISO13485 and ISO 9001 certified facility
- Defined medium – enhances reproducibility
- Superior cell expansion
- Maintains trilineage differentiation potential through long term passaging



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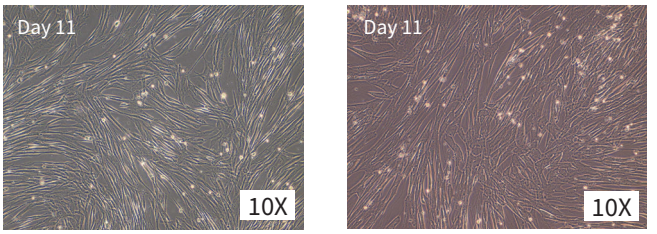
Isolation Potential

STEMin1™ MSC-SFM supports successful isolation of MSCs from various human tissues such as Wharton's jelly, bone marrow, adipose tissue and dental pulp.

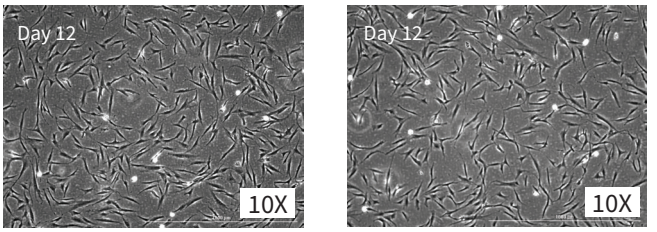
Isolation of MSCs from Wharton's Jelly



STEMin1™ | 10% FBS containing medium

Fig 1 : MSCs were isolated from human Wharton's Jelly in STEMin1™ and serum containing medium. Confluence is attained between Day 10-15 post-isolation.

Isolation of MSCs from Adipose Tissue



STEMin1™ | 10% FBS containing medium

Fig 2 : MSCs were isolated from human lipoaspirate in STEMin1™ and serum containing medium. Confluence is attained between Day 10-13 post-isolation.

Superior yield of WJ-MSCs post isolation from multiple cord tissues

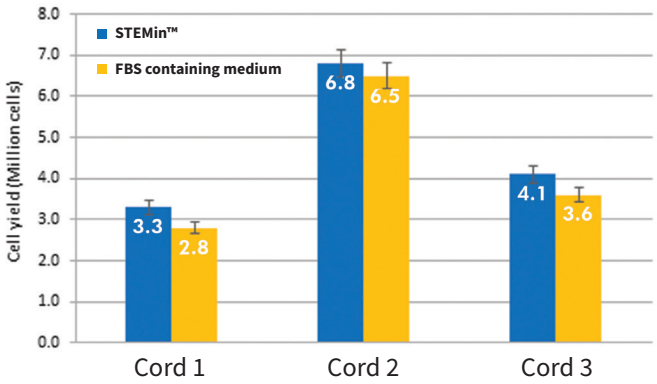


Fig 3 : MSC yield in STEMin1™ from 3 different human cords

Superior Expansion Capacity

STEMin1™ MSC-SFM supports self-renewal of MSCs while maintaining their undifferentiated state and multipotency.

Supports high cell densities

STEMin1™ MSC-SFM supports higher cell densities in comparison with FBS supplemented medium through long term passaging (up to 5 passages).

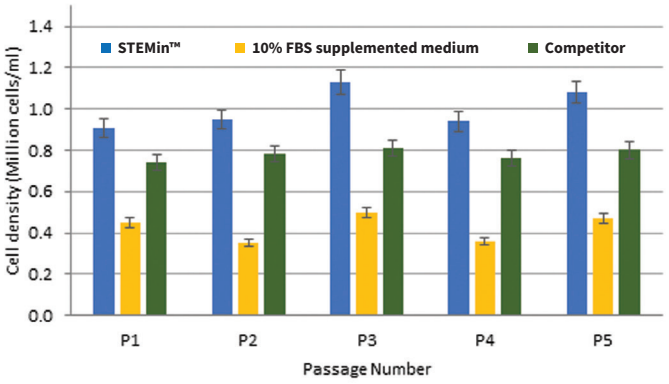


Fig 4 : Viable cell densities of WJ-MSCs cultured in STEMin1™ MSC-SFM for 5 passages.

Faster growth and less doubling time

STEMin1™ MSC-SFM enhances cell growth rates resulting in less doubling time.

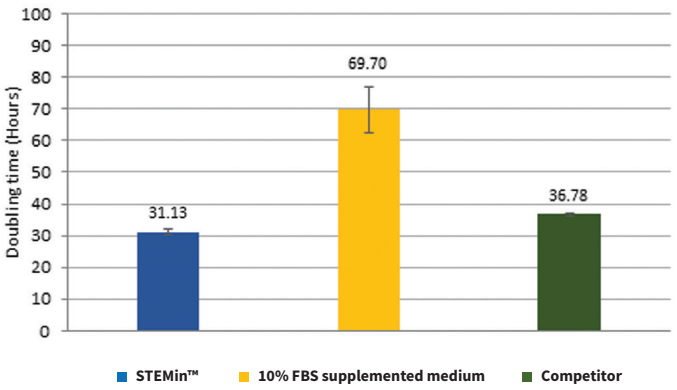
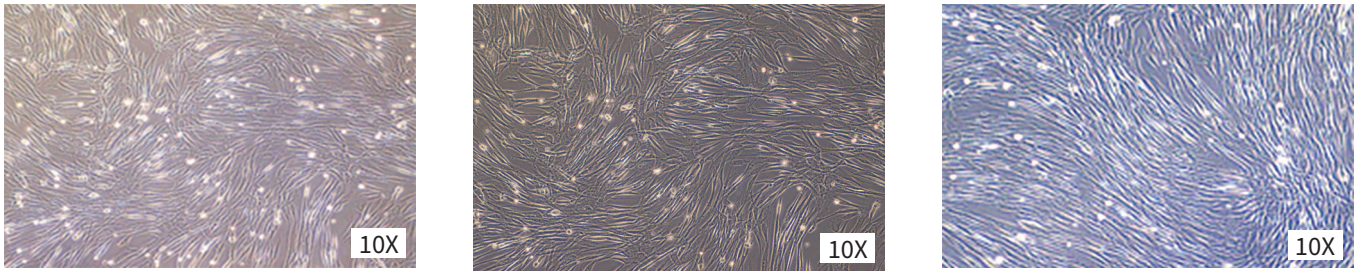


Fig 5 : Doubling time of WJ-MSCs in STEMin1™ MSC-SFM

Ideal Morphology

STEMin1™ MSC-SFM maintains MSCs in their spindle shaped, fibroblast like morphology post-isolation as well as during serial subculturing.



STEMin1™ | 10% FBS supplemented medium | Competitor MSC-SFM

Fig 6: WJ-MSCs were expanded in each medium for 5 subcultures in T25 flasks. STEMin1™ and competitor MSC-SFM showed healthy spindle shaped fibroblastic morphology. Morphology was maintained in STEMin1™ and competitor medium through 5 subcultures.

Assured Purity

According to the standard criteria established by The international Society for Stem Cell Research and Therapy, >95% MSC population must express stem-ness markers - CD90 and CD105 and must lack expression of hematopoietic markers - CD34 and CD45. STEMin1™ maintains stem-ness of isolated and cultured MSCs by retaining surface marker epitopes.

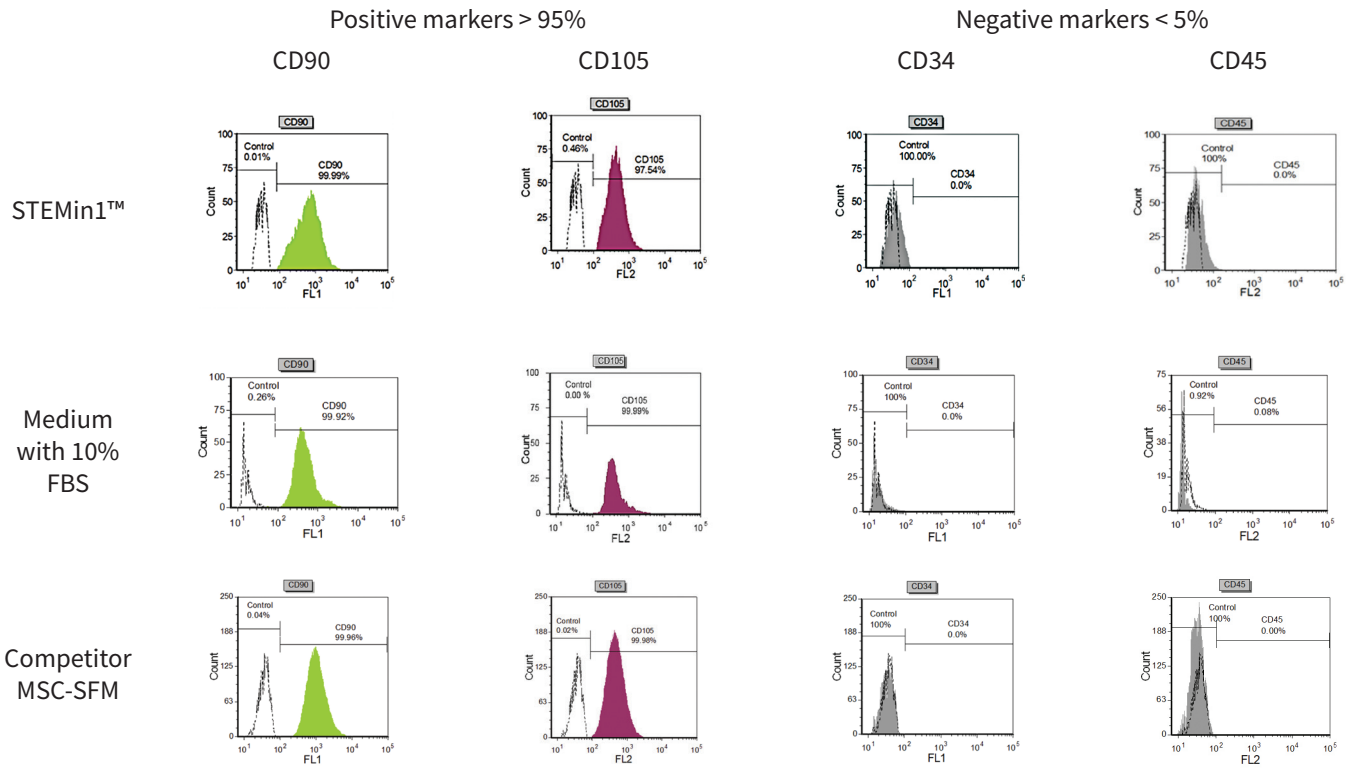


Fig 7: Immunophenotyping of WJ-MSCs cultured in each medium

Differentiation Potential

Ability for trilineage differentiation is one of the main characteristics of stem-ness of stem cells. MSCs isolated and cultured in STEMin1™ MSC-SFM are successfully differentiated into adipocytes, osteocytes and chondrocytes using respective differentiation medium and staining method.

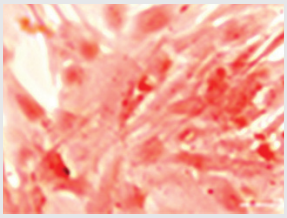
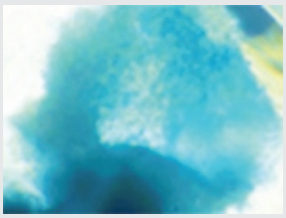
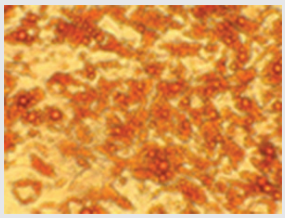
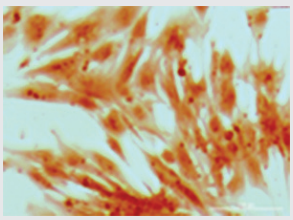
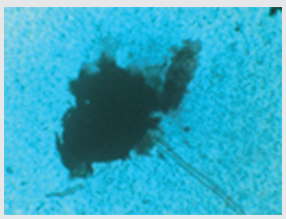
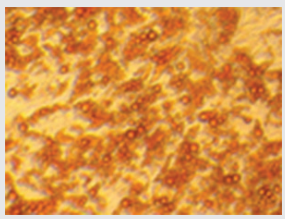
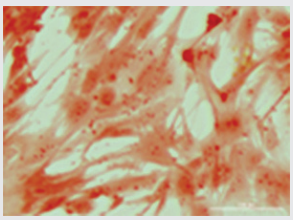
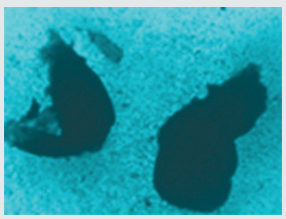
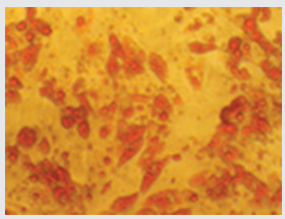
	A	B	C
STEMin1™			
Medium with 10% FBS			
Competitor MSC-SFM			

Fig 8: WJ-MSCs were cultured in STEMin1™, 10% FBS supplemented medium and competitor MSC-SFM for 5 subcultures followed by trilineage differentiation.
A: Osteocytes stained with Alizarin Red S
B: Chondrocytes stained with Alcian blue
C: Adipocytes stained with Oil-Red-O

Preparation of complete STEMin1™

1. Thaw STEMin1™ growth supplement (Part B) overnight at 2-8°C.
   Note: Few precipitates in Part B may be visible after thawing. Precipitates will not affect the performance of the medium.
2. Disinfect the external surface of the bottles of Part A **(500ml)** and Part B**(60ml)** by spraying with isopropyl alcohol before placing in a biosafety hood.
3. Discard ~**60ml** of Part A. Transfer the entire content of Part B **(60ml)** to basal medium Part A **(500ml)** under aseptic condition.
   Note: If desired, 1ml of antibiotic-antimycotic solution (A002) can be added to 100ml of complete medium.
4. Tightly cap the bottle and swirl gently to ensure proper mixing.
   Note: Do not mix vigorously. Doing so will cause formation of foam.
5. Tightly cap the bottle and swirl gently to ensure proper mixing.
   Note: Do not mix vigorously. Doing so will cause formation of foam.

Important Note:
For initial isolation of MSCs it is recommended to add ~ 2.5 - 5% human AB serum to the complete medium to facilitate cell attachment and growth till culture reaches confluence at P0 (Requirement of human AB serum may be varied between different MSC sources). After P0, they can be cultured in complete medium without supplementation of Human AB serum.

Storage and shelf life

1. Store STEMin1™ Part A at 2-8°C away from bright light.
2. Store STEMin1™ Part B at -20°C.
3. Store the reconstituted medium at 2-8°C and use it within 3 – 4 weeks. Do not freeze it.
4. Use before expiry date given on the product label.
   Note: Do not freeze the basal medium.
   Avoid repeated freezing and thawing of the growth supplement.

STEMin1™ Attachment Solution

A Defined, Serum free (SF), Xeno-free (XF) attachment solution for MSCs

- Ready-to-use substrate for attachment and expansion of human MSCs
- Animal component - free and Xeno-free
- Efficient attachment and spreading

Surface coating of culture vessel

1. Thaw the bottle of STEMin1™ Attachment Solution and bring it to room temperature.
2. Aseptically add this solution to the culture vessel as per recommended volumes mentioned in Table 1.
3. Incubate the vessel overnight at 37°C.
   Note: For uniform coating, make sure that the incubator is properly levelled.
4. Aspirate the solution with the help of pipette.
5. If vessel is not used immediately, store the coated vessel at 2 – 8°C up to one week.
   Note: Flask should be kept with caps tightly closed and plates should be sealed with a parafilm during storage.

Table 1 : Recommended volumes of STEMin1™ Attachment Solution for different culture vessels

Culture vessel	Volume
96 well plate	100µl per well
48 well plate	150µl per well
24 well plate	300µl per well
12 well plate	500µl per well
6 well plate	1ml per well
T25 flask	2 – 3ml per flask
T75 flask	5 – 6ml per flask

Ordering information

Product Name	Packing
STEMin1™ Attachment Solution	TCL206-10ML

STEMin1™ Dissociation Solution

A Defined, Serum free (SF), Xeno-free (XF) dissociation solution for MSCs

- Gentle on cells : Dissociates the cells within a minute without affecting viability
- Retention of marker expression : Maintains cell surfece epitopes unalterd

Seeding, maintenance and culturing of cells

1. Recommended seeding density in STEMin1™ is 15000 cells per cm^2. Refer the table mentioned below.

Culture vessel	Recommended seeding density	Total No. of cells per flask	Volume of medium
T12.5 flask	15000 cells / cm^2	0.2×10^6	3 – 4ml per flask
T25 flask		0.4×10^6	5 – 6ml per flask

2. Monitor the cells every day for cell morphology and health.
3. Change the medium every alternate day.
4. Subculture once the cells reach 70 – 80% confluence.
5. Aspirate the medium and discard. Do not disturb the monolayer.
6. Wash the cells with sufficient volume of DPBS to remove residual medium.
7. Aspirate it off and discard.
8. Add appropriate volume of STEMin1™ Dissociation Solution in the culture vessel. Refer table 2.
   Table 2 : Recommended volumes of Dissociation Solution as per culture vessel.

Culture vessel	Recommended volume of STEMin1™ Disociation Solution
T12.5	0.3ml
T25	0.5ml
T75	1.5ml
T175	3ml

9. Gently rock the flask to ensure complete coverage of dissociation solution over the cells.

10. Incubate the flask at 37°C. Complete dissociation requires 1 – 2 minutes.
11. Microscopically observe the flask at regular intervals. When the cells start rounding up, gently tap the flask to ensure complete detachment of cells from surface.
12. Add equal volume of STEMin1™ Neutralizer.
13. Pipette gently to get a homogenous cell suspension.
14. Centrifuge at 600rpm for 2 – 3 minutes.
15. Discard supernatant and suspend the pellet in 3ml fresh complete medium.
16. Count cells using hemocytometer.
17. Seed at recommended seeding density in a new flask coated with attachment solution and containing fresh complete medium.

Ordering information

Product Name	Packing
STEMin1™ Dissociation Solution	TCL208-100ML
STEMin1™ Neutralizer	TCL209-100ML

FREEZin1™ Universal freezing medium

A xeno-free, serum-free cryopresevation medium for MSCs

- High recovery rates
- More than 90% post-revival viability
- Optimized for MSCs cultured in STEMin1™
- Maintains trilineage differentiation potential
- Ready-to-use

Cryopreservation

1. Harvest cells from the flask when they are 80-90% confluent.
2. To harvested cell pellet add cold cell freezing medium (TCL098) at approximately 0.5 x 10^6 – 1 x 10^6 cells /ml.
3. Aliquot cells in appropriate cryogenic storage vials. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Alternatively, place the cryovials containing the cells in an isopropanol
4. Chamber and store them at -80°C overnight. Alternatively, store them at -20°C for 1 - 2 hours before shifting to -80°C overnight.
5. Transfer cryovials to liquid nitrogen tank for long term storage.

Revival

1. Add 5ml complete medium to the coated T25 flask.
2. Place the flask at 37°C to equilibrate the medium.
3. Remove the cryovial from liquid nitrogen tank wearing appropriate personal protective equipment.
4. Hold the cryopreserved vial in water bath set at 37°C and let it thaw partially.
   Note: DO NOT hold the vial in water bath for more than 90-120 seconds. AVOID getting water up to the cap of the vial.
5. Disinfect the vial by swabbing thoroughly with 70% isopropyl alcohol.
6. Add the cell suspension drop by drop to the T25 flask containing the pre-warmed complete medium. Keep swirling the flask while adding the cell suspension.
   Note: Dropwise addition is required to prevent the cells from stress induced by exothermic reaction.
7. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium.
8. Incubate the cells at 37°C and 5% CO_2.
9. Check for cell attachment in 2-3 hours.
10. If more than 70-80% cells are attached, replace the medium with fresh medium.
    Note: Medium change after 2-3 hours is mandatory to remove traces of DMSO.
11. If cells have not attached, centrifuge the cell suspension at 600 rpm for 7-8 minutes and resuspend in fresh medium
12. Incubate the cells at 37°C and 5% CO_2.

Ordering information

Product Name	Packing
FREEZin1™ Universal freezing medium	TCL098-50ML