Human mesenchymal stem cell protocol: subculturing hMSCs

HyClone media and supplements

Procedure

Overview

Human Mesenchymal Stem Cells (hMSCs) are primary cells that can be successfully cultured approximately eight passages. This procedure has been supplied to provide a protocol for the subculturing of these cells.

The procedure is adapted from Kamath, A., Cellular Engineering Technologies, Inc (1).

Required materials

- Complete hMSC expansion medium (Table 1)
- hMSCs (Table 2)
- HyClone™ ES Qualified DPBS (SH30850.03)
- HyClone HyQTase™ (SV30030.01) or Trypsin (SH30042.01)
- General cell culture supplies

This product is intended for research use only.

Medium preparation

Prepare complete expansion medium according to Table 1. Available hMSCs are listed in Table 2.

Table 1. Complete hMSC expansion medium, 500 mL

HyClone AdvanceSTEM Mesenchymal Stem Cell Expansion Kit (SH30875.KT)	Volume (500 mL final)	Product code
AdvanceSTEM Mesenchymal Stem Cell Basal Medium	450 mL	SH30879.02
AdvanceSTEM Cell Growth Supplement	50 mL (10%)	SH30878.01

Table 2. Available human mesenchymal stem cells

Cell description	Size	Volume	Product code
CET Human Adipose-Derived Mesenchymal Stem Cells	2.0 mL vial	≥ 100 000 cells per mL	SV30102.01
		≥ 500 000 cells per mL	SV30102.02
CET Human Amniotic Mesenchymal Stem Cells	2.0 mL vial	≥ 100 000 cells per mL	SV30103.01
		≥ 500 000 cells per mL	SV30103.02
CET Human Bone Marrow Mesenchymal Stem Cells	2.0 mL vial	≥ 100 000 cells per mL	SV30110.01
		≥ 500 000 cells per mL	SV30110.02
CET Human Cord Blood CD34+ Stem Cells	2.0 mL vial	≥ 100 000 cells per mL	SV30106.01
		≥ 500 000 cells per mL	SV30106.02
		≥ 1 000 000 cells per mL	SV30106.03
CET Human Cord Blood CD133+ Stem Cells	2.0 mL vial	≥ 100 000 cells per mL	SV30107.01
		≥ 500 000 cells per mL	SV30107.02
		≥ 1 000 000 cells per mL	SV30107.03

General considerations

Any unused AdvanceSTEM™ Stem Cell Growth Supplement should be aliquoted and refrozen.

Store all media at 2°C to 8°C and avoid extended exposure to room or higher temperatures. Equilibrate all media in a water bath set at 37°C before adding media to any cell culture.

Antibiotics / antimycotics should not be used as an alternative to proper aseptic technique. However, should you prefer to add antibiotics to your formulation, a concentration of 10 mL/L HyClone Pen/Strep/Fungizone (SV30079.01) is appropriate.

Protocol

Step	Action
1	In the laminar flow hood, remove spent medium from cell monolayer and discard.
2	Wash the monolayer with DPBS ES Qualified (SH30850.03) by adding 10 mL/75 cm^2 to the flask, being careful not to disturb the monolayer. Rock the flask back and forth. Remove the DPBS from the monolayer and discard.
3	Add trypsin (SH30042) or HyQTase (SV30030.01) at 3 to 5 mL/75 cm^2 flask and rock the flask to ensure the entire monolayer is covered with the solution.
4	If using trypsin, incubate at 37°C until the hMSCs begin to detach (approximately 5 min). If using HyQTase, use at room temperature until the hMSCs begin to detach (approximately 5 min). Do not exceed 15 min. Care should be taken that the cells not be forced to detach prematurely, as this can result in clumping.
5	Add complete hMSC expansion medium (Table 1) in equal amounts to trypsin or HyQTase and pipette the cells up and down until the cells are dispersed into a single cell suspension.
6	To remove the trypsin or HyQTase, centrifuge cells for approximately 10 min at room temperature. Aseptically remove supernatant.
7	Resuspend the cell pellet in prewarmed complete hMSC expansion medium (Table 1) at approximately 5 mL/pellet from 75 cm^2 flask. Remove a small volume sample for counting.
8	Count the cells with a hemacytometer or cell counter and calculate cell count.

Step	Action
9	Seed new flasks at 5000 to 6000 cells/cm^2 by adding the appropriate volume of cell suspension into fresh prewarmed complete hMSC expansion medium (Table 1).
10	Incubate cells at 37°C in 5% CO_2 and 90% humidity.
11	Perform a medium exchange 3 to 4 days after subculture, by replacing spent medium with equal volumes of fresh hMSC expansion medium (Table 1). Cultures should be ready to subculture every five to seven days.

Reference

1. Kamath, A., Cellular Engineering Technologies, Inc., *http://celleng-tech.com*

Related procedures

Procedure: *Human mesenchymal stem cell protocol: cryopreservation*, 29154512.

Procedure: *Human mesenchymal stem cell and multipotent cord blood unrestricted somatic stem cell protocol: thawing and plating*, 29154590.

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