

# Human Mesenchymal Stem Cell Protocol: Chondrogenic Differentiation

Protocol  
SC 00012

*For research use only*

## Background

Thermo Scientific HyClone AdvanceSTEM Chondrogenic Differentiation Kit (SH30885.KT) has been developed to support the chondrogenic differentiation of human mesenchymal stem cells isolated from adipose tissue and bone marrow. Chondrogenic differentiation should be done in pellet culture and takes 28 days.

## Required Materials

- Thermo Scientific HyClone AdvanceSTEM Complete Mesenchymal Stem Cell Expansion Media
- Thermo Scientific HyClone AdvanceSTEM Complete Chondrogenic Kit
- Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03)
- Thermo Scientific HyClone Trypsin (SH30042.01)

## Media Preparation

Table 1: 500 mL Complete HyClone AdvanceSTEM hMSC Expansion Medium

Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM™ Mesenchymal Stem Cell Basil Medium	450 mL	SH30879.02 (1000 mL)
AdvanceSTEM Cell Growth Supplement	50 mL (10%)*	SH30878.01
Store at 2-8°C. Discard unused medium after 8 weeks.		

Table 2: 500 mL Complete HyClone AdvanceSTEM Chondrogenic Kit (SH30889.02)

Thermo Scientific HyClone Product	Volume (500 mL final)	Catalog Number
AdvanceSTEM Chondrogenic Differentiation Medium	450 mL	SH30889.02 (450 mL)
Store at 2-8°C. Discard unused medium after 2 weeks.		

*Note: Antibiotics should not be used as an alternative to proper aseptic technique. However, should you prefer to add antibiotics to your medium use [1%] of Thermo Scientific HyClone Pen/Strep (SV30079.01).*

## Protocol

1. In a laminar flow hood, pipette spent medium from the cell monolayer and discard.
2. Wash the monolayer with HyClone ES-Qualified DPBS.  
Use 10 mL / T-75cm<sup>2</sup>. Rock the flask gently then remove the DPBS and discard.
3. Add Trypsin at 5 mL / T-75cm<sup>2</sup>. Rock the flask to spread the Trypsin across the entire monolayer. Incubate at 37°C until the cells begin to detach. This should take approximately 5 minutes but no more than 15 minutes. Care should be taken that the cells are not forced to detach prematurely, as this may result in clumping.
4. Inactivate the Trypsin by adding at least an equal volume of complete AdvanceSTEM Mesenchymal Stem Cell Expansion Media (Table 1). Pipette the cells up and down to further separate into a single cell suspension.
5. Resuspend the cells in a conical tube and centrifuge at 200 x g for 10 minutes. Remove supernatant.

6. Resuspend the cells in complete AdvanceSTEM Chondrogenic Differentiation Media (Table 2). Remove a small sample volume for counting.
7. Count the cells with a hemacytometer or cell counter and calculate desired number.  $1 \times 10^6$  cells per pellet is recommended.
8. Centrifuge cells at  $200 \times g$  for 10 minutes to pellet. Gently remove the supernatant.
9. Carefully add 5 mL fresh AdvanceSTEM Chondrogenic Differentiation Media (Table 2).
10. Loosen the cap of the conical tube with the pellet and incubate at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ .
11. Every 3 days remove all media and replace with fresh complete HyClone AdvanceSTEM Chondrogenic Differentiation Medium (Table 2). Be careful to never disturb the pellet. Chondrogenesis takes 28 days.

Related Protocols

- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Sub Culturing hMSCs
- SC Protocol 00010 - Human Mesenchymal Stem Cell Protocol: Adipogenic Differentiation

©2009 Thermo Fisher Scientific Inc. All rights reserved.