

# Human Mesenchymal Stem Cell Protocol: Chondrogenic Differentiation

Adapted from Kamath, A., *Cellular Engineering Technologies, Inc.*

Thermo Scientific HyClone Chondrogenic Differentiation Kit has been designed to support the chondrogenic differentiation of human Mesenchymal Stem Cells isolated from bone marrow and adipose tissue.

Chondrogenic differentiation should always be done in a pellet culture. To create a culture for chondrogenesis, trypsinize  $1 \times 10^6$  mesenchymal stem cells.

*Note: Store all media at 2-8°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.*

## Thawing Cells

1. In a laminar flow hood, pipette spent medium from cell monolayer and discard spent medium.
2. Wash the monolayer with Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03) by adding 10mL/75cm<sup>2</sup> 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 Thermo Scientific HyClone Trypsin (SH30042.01) at 3-5 mL/75 cm<sup>2</sup> flask and rock the flask to ensure that the entire monolayer is covered with the trypsin solution.
4. Incubate at 37°C until the cells begin to detach (approximately 5 minutes). **Do not exceed 15 minutes.** Care should be taken not to force the cells to detach prematurely, as this may result in clumping.
5. To remove the trypsin, add an equal volume of complete Chondrogenic Differentiation medium. (Table 1).
6. Spin the cells for 10 minutes at 200 x g in a centrifuge. A swing bucket rotor is required since the cells will pellet to the bottom of the tube rather than on the side.
7. Once the cells are pelleted, gently aspirate the supernatant, leaving the pellet behind.
8. Add 4 ml of fresh complete Chondrogenic Differentiation medium (Table 1) on top of the pellet but do not disturb the pellet or resuspend in solution.
9. Make sure that the cap on top of the conical tube is loosely fitted.

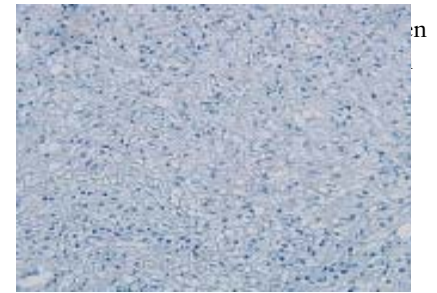


Figure 1: Cells stained for cartilage using toluidine blue staining.

11. Replace media every 3 days. Be careful not to disturb your pellet when withdrawing the media. Again, gently add media to the pellet. Do not resuspend or disturb the pellet using a pipette etc.
12. Chondrogenesis takes 28 days and can be visualized by staining and microscopy.

*Note: 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 per liter is appropriate. Use Thermo Scientific HyClone Pen/Strep/Fungizone, SV30079.01*

Table 1: Preparation of 500 mL complete Chondrogenic Differentiation Medium

Thermo Scientific HyClone AdvanceSTEM™ Chondrogenic Differentiation Kit (SH30885.KT)			
Brand	Amount for 500 mL	HyClone Product	Catalog #
Thermo Scientific	450 mL	AdvanceSTEM Chondrogenic Differentiation Medium	SH30889.02 (450 mL)
Thermo Scientific	50 mL	AdvanceSTEM™ Stem Cell Growth Supplement	SH30878.01 (50 mL)

Store at 2-8°C. Discard unused medium after 8 weeks.

*In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.*

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