

Chondrogenic Differentiation Medium

Catalog Number: CIHM-MED5

Volume: 500 mL

Product Type: Ready-to-use, serum-free chondrogenic induction medium

Compatibility: For use with CIHM001 after SV40 LT silencing (withdrawal of doxycycline)

Product Description

CIHM-MED5 is a sterile, serum-free, chemically defined complete medium formulated to induce the chondrogenic differentiation of Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells (Catalog #CIHM001). This medium is intended for use following the withdrawal of doxycycline (using CIHM-MED2), which silences SV40 Large T antigen expression and allows CIHMSCs to exit the proliferative state and recapitulate primary MSC differentiation capacity.

CIHM-MED5 contains specific growth factors and non-essential amino acids to support mesenchymal condensation, extracellular matrix deposition (collagen type II and aggrecan), and formation of cartilage-like pellets.

Note: This medium is serum-free, doxycycline-free, and puromycin-free. Do not supplement with FBS, as serum inhibits chondrogenesis in micromass/pellet culture.

Physical and Chemical Properties

Parameter	Specification
Appearance	Clear, pink to slightly orange-red solution
pH	7.2 – 7.4 (at 25°C)
Osmolality	320 – 360 mOsm/kg
Sterility	0.1 µm filtered, sterile
Endotoxin level	< 0.1 EU/mL (critical for growth factor integrity)
Protein content	Low (BSA only from ITS+, no serum)
Storage temperature	-20°C or -80°C (for long-term) or 2–8°C (short-term, see Section 6)
Shelf life (unopened)	6 months at -20°C; 4 weeks at 2–8°C

Usage Instructions

Preparation Before Differentiation

Before using CIHM-MED5, CIHMSCs must be switched from expansion conditions to a quiescent state:

Step	Procedure
1. Expand	Grow CIHM001 in CIHM-MED1 (with doxycycline + puromycin) to ~80% confluence
2. Wash	Aspirate medium, wash twice with PBS (no Ca^{2+} / Mg^{2+})
3. SV40 LT silencing	Replace with CIHM-MED2 (Doxycycline Withdrawal Medium) for 48–72 hours
4. Harvest	Detach cells with Trypsin-EDTA, count, and proceed to chondrogenic culture

Chondrogenic Differentiation Protocol (Pellet Culture – Recommended)

- 1. Prepare cell suspension:** Resuspend silenced CIHMSCs in CIHM-MED5 at **2.5×10^5 cells per 1 mL**.
- 2. Form pellets:** Aliquot 0.5 mL of cell suspension (1.25×10^5 cells) into a 15 mL conical tube. Centrifuge at $500 \times g$ for 5 minutes at room temperature to form a loose pellet.
- 3. Incubate:** Loosen caps for gas exchange. Incubate at 37°C , 5% CO_2 , humidified atmosphere.
- 4. Medium change:** Gently aspirate 0.4 mL of supernatant every 2–3 days and replace with fresh pre-warmed CIHM-MED5 (do not disturb pellet).
- 5. Duration:** 14–28 days. Pellets become opaque, spherical, and firm by day 14.

Alternative: Micromass Culture

- Seed 2×10^5 cells in a 10 μ L droplet per well of a 24-well plate. Allow 2 hours for attachment (37°C, 5% CO₂), then gently add 0.5 mL CIHM-MED5 per well. Change medium every 2–3 days.

Differentiation Validation Markers

After 21–28 days in CIHM-MED5, CIHMSCs should demonstrate the following chondrogenic features:

Assay / Marker	Expected Result
Alcian Blue stain (pH 1.0)	Intense blue staining of sulfated proteoglycans in pellet sections
Toluidine Blue O	Metachromatic purple-red staining of glycosaminoglycans
Collagen Type II (immunostaining)	Strong pericellular and extracellular positivity
Aggrecan (immunostaining)	Diffuse extracellular matrix staining
Collagen Type I (negative control)	Minimal or no staining
Safranin O	Bright orange-red staining in pellet sections
qPCR (vs. undifferentiated CIHMSCs)	Upregulation of SOX9, ACAN, COL2A1 (> 50-fold); downregulation of COL1A1
Pellet diameter (Day 21)	> 1.5 mm from starting 0.5 mm pellet

Storage and Handling

Condition	Instruction
Upon receipt	Store at -20°C or -80°C immediately (growth factors are unstable at 4°C long-term)
Thawing	Thaw overnight at 2–8°C. Do not heat in a 37°C water bath
After thawing	Can be stored at 2–8°C for up to 4 weeks if used frequently. Aliquot if storing longer.
Aliquoting	Dispense into sterile single-use aliquots (e.g., 50 mL) to avoid repeated freeze-thaw cycles.
Light sensitivity	Protect from light (store in opaque or foil-wrapped containers)
Do not refreeze once thawed for more than 24 hours.	

Quality Control

Each batch of CIHM-MED5 is tested for:

Test	Acceptance Criteria	Method
Sterility	No growth	14-day USP <71>
Mycoplasma	Negative	PCR
Endotoxin	< 0.1 EU/mL	LAL chromogenic
Chondrogenic activity	Alcian Blue positive pellets with CIHM001 after 21 days	Bioassay
Growth factor integrity	Active TGF- β 3 and BMP-2 (ELISA confirmed)	Sandwich ELISA

Troubleshooting Guide

Problem	Possible Cause	Solution
Pellets do not form	Insufficient centrifugation or low cell number	Use $1.25\text{--}2.5 \times 10^5$ cells/pellet; centrifuge at $500 \times g$ for 5 min
Pellets dissociate	Incomplete SV40 LT silencing (residual doxycycline)	Extend CIHM-MED2 treatment to 72 hours

Problem	Possible Cause	Solution
Poor matrix staining	TGF- β 3 degradation	Use fresh aliquots; avoid multiple freeze-thaws
Cell death	FBS contamination	Ensure no FBS added; only use serum-free CIHM-MED5
Fibroblastic morphology (no chondrogenesis)	BMP-2 absent or inactive	Validate growth factor lot; supplement fresh BMP-2 at 10 ng/mL

Limitations and Warnings

Category	Statement
Intended use	For research use only. Not for human therapeutic, diagnostic, or veterinary use.
Growth factors	Human cytokines. Handle with care.
Sterility	Do not use if turbid or if precipitate appears after thawing.
Serum-free	Do not supplement with serum (inhibits chondrogenesis).
Doxycycline	This medium does NOT contain doxycycline. Use only after SV40 LT silencing.
Biosafety	No BSL restrictions for medium alone; however, start with BSL-2 cells (CIHM001).

Ordering Information

Product	Catalog #	Volume
Chondrogenic Differentiation Medium	CIHM-MED5	500 mL
Complete Expansion Medium (Dox + Puro)	CIHM-MED1	500 mL
Doxycycline Withdrawal Medium	CIHM-MED2	500 mL
Osteogenic Differentiation Medium	CIHM-MED3	500 mL
Adipogenic Differentiation Medium	CIHM-MED4	500 mL
Chondrogenic Supplement Pack	CIHM-ADD5	1 vial each

Storage and Stability Summary

Condition	Duration
Unopened, -20°C, dark	6 months
Unopened, -80°C, dark	12 months
Thawed, 2–8°C, dark	4 weeks (once)
Thawed and aliquoted, -20°C	3 months (avoid >2 freeze-thaw cycles)
37°C water bath	Not recommended