



Data Sheet

Complete Expansion Medium

Catalog Number: CIHM-MED1

Volume: 500 mL

Product Type: Ready-to-use complete cell culture medium

Compatibility: For use with CIHM001 (Conditionally Immortalized Human BM-MSCs)

Product Description

CIHM-MED1 is a sterile, ready-to-use complete culture medium specifically formulated for the optimal expansion of Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells (Catalog #CIHM001). The medium contains doxycycline to induce SV40 Large T antigen (SV40 LT) expression via the TetON system, enabling robust proliferation, and puromycin to maintain selective pressure for stable transgene integration.

This medium preserves the characteristic spindle-shaped, fibroblast-like morphology of CIHMSCs and maintains expression of key MSC surface markers (CD73+, CD90+, CD105+) during expansion.

Physical and Chemical Properties

Parameter	Specification
Appearance	Clear, pink to slightly orange-red solution
pH	7.2 – 7.4 (at 25°C)
Osmolality	290 – 320 mOsm/kg
Sterility	0.1 µm filtered, sterile
Endotoxin level	< 0.5 EU/mL
Storage temperature	2°C to 8°C, protected from light
Shelf life (unopened)	6 months from the date of manufacture

Usage Instructions

Thawing and Plating CIHMSCs (First Use)

1. Remove CIHM-MED1 from 4°C storage. Do not heat-inactivate.
2. Warm to 37°C in a water bath for 15–20 minutes, or overnight at 4°C.
3. Thaw one vial of CIHM001 (500,000 cells) rapidly in a 37°C water bath (1–2 minutes).
4. Transfer cells dropwise into 10 mL of pre-warmed CIHM-MED1 in a 15 mL conical tube.
5. Centrifuge at $300 \times g$ for 5 minutes at room temperature.
6. Aspirate supernatant and resuspend pellet in 5 mL of fresh CIHM-MED1.

7. Transfer to a T25 flask or one well of a 6-well plate. Incubate at 37°C, 5% CO₂, humidified atmosphere.

Routine Subculture

Step	Instruction
Confluence for passage	80–90%
Medium renewal	Every 2–3 days
Trypsinization	Cell Detach Solution (3–5 min at 37°C)
Neutralization	Add equal volume of CIHM-MED1
Split ratio	1:3 to 1:5
Final seeding density	5,000 – 10,000 cells/cm ²

Switching to Differentiation Conditions

To induce differentiation, **do not use CIHM-MED1**. Instead:

1. Wash cells twice with PBS.
2. Replace with CIHM-MED2 (Doxycycline Withdrawal Medium, Cat# CIHM-MED2) for 48–72 hours to silence SV40 LT expression.
3. Then replace with standard MSC differentiation media (osteogenic, adipogenic, chondrogenic).

Performance Validation



Test	Acceptance Criteria	Result
Cell doubling time (CIHM001)	24–36 hours	Pass
Viability after 5 passages	>90%	Pass
Marker retention	CD73+/CD90+/CD105+ >90%	Pass
Puromycin resistance	>95% cells viable after 3 days in CIHM-MED1	Pass
Doxycycline inducibility	>10-fold SV40 LT expression vs. no doxycycline	Pass
Sterility	Negative for bacteria, fungi	Pass
Mycoplasma	Negative (PCR)	Pass

Important Handling Notes

- **Do not freeze** CIHM-MED1. Freezing causes precipitation and loss of activity.
- **Protect from light** – Doxycycline is light-sensitive. Store in original opaque or amber bottle. If not supplied in light-protected container, wrap with foil.
- **Do not use** with standard FBS (contains tetracycline, causing basal expression). This product contains tetracycline-free FBS.
- **Do not heat-inactivate** FBS – heat inactivation can reduce performance.
- **Pre-warm only once** – Repeated heating cycles degrade doxycycline and puromycin.
- **Use within 4 weeks** after first opening. Discard if color changes to orange/yellow (indicating pH drop) or if visible precipitate forms.



Quality Control

Each batch of CIHM-MED1 is tested for:

- Sterility (14-day test per USP <71>)
- Mycoplasma (PCR)
- Endotoxin (LAL assay)
- Cell growth promotion (using CIHM001, 5 passages)
- Absence of cytotoxicity

Limitations and Warnings

Category	Statement
Intended use	For research use only. Not for human therapeutic, diagnostic, or veterinary use.
Safety	Puromycin is toxic. Avoid skin contact. Use appropriate PPE (gloves, lab coat, eye protection).
Disposal	Dispose as biohazardous waste. Medium may contain trace antibiotics and selection agents. Follow institutional guidelines.
Biosafety	CIHM-MED1 itself is not infectious, but it is intended for BSL-2 cells (CIHM001). Handle accordingly.



Ordering Information

Product	Catalog #	Volume
Complete Expansion Medium (Dox + Puro)	CIHM-MED1	500 mL
Doxycycline Withdrawal Medium	CIHM-MED2	500 mL
Puromycin Solution (1 mg/mL)	CIHM-SEL1	1 mL
Doxycycline Solution (2 mg/mL)	CIHM-SEL2	1 mL
Trypsin-EDTA (0.25%)	CIHM-REAG1	100 mL

Storage and Stability Summary

Condition	Duration
Unopened, 2–8°C, dark	6 months
Opened, 2–8°C, dark	4 weeks
37°C water bath	<30 minutes (single use)
Repeated warming	Not recommended
Freezing (-20°C or -80°C)	Not allowed

IMPORTANT: This warranty is valid for one month from the original purchase date.