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Data Sheet

Osteogenic Induction Medium

Catalog Number: CIHM-MED2B
Volume: 500 mL
Product Type: Complete, ready-to-use osteogenic differentiation medium
Compatibility: For use with CIHM001 (Conditionally Immortalized Human BM-MSCs) after doxycycline withdrawal

Product Description

CIHM-MED2B is a sterile, complete osteogenic induction medium specifically formulated to drive differentiation of Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells (Catalog #CIHM001) toward the osteoblast lineage. This medium contains a standardized formulation of ascorbic acid (ascorbate), β-glycerophosphate, and dexamethasone, the three essential components for osteogenic differentiation.

For optimal results, CIHMSCs must first be cultured in **CIHM-MED2 (Doxycycline Withdrawal Medium)** for 48–72 hours to silence SV40 Large T antigen expression before switching to CIHM-MED2B. This ensures terminal differentiation without uncontrolled proliferation.

Differentiated cells demonstrate mineralized extracellular matrix, detectable by Alizarin Red S or von Kossa staining, and express osteoblast-specific markers including alkaline phosphatase (ALP), osteocalcin (OCN), and runt-related transcription factor 2 (RUNX2).

Note: This medium is **serum-containing** (10% FBS) and **does NOT contain** doxycycline or puromycin. It is intended for use after transgene silencing.

Physical and Chemical Properties

Parameter	Specification
Appearance	Clear, pink to slightly orange-red solution
pH	7.2 – 7.4 (at 25°C)
Osmolality	300 – 340 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)	3 months from date of manufacture
Shelf life (opened)	2 weeks (if stored at 2–8°C and protected from light)

Important: Ascorbic acid and dexamethasone are light- and temperature-sensitive. Do not warm repeatedly.

Usage Instructions

Before Starting – Transgene Silencing

Do NOT add CIHM-MED2B directly to cells maintained in CIHM-MED1 (Dox + Puro medium). First, silence SV40 LT expression:

1. Expand CIHM001 in CIHM-MED1 until 70–80% confluence.
2. Wash cells twice with PBS.
3. Replace with **CIHM-MED2 (Doxycycline Withdrawal Medium)** for 48–72 hours. Change medium every 24 hours.

4. After silencing, proceed to osteogenic induction.

Osteogenic Differentiation Protocol

Step	Instruction
Seeding density	5,000 – 10,000 cells/cm^2 in CIHM-MED2 (withdrawal medium)
Day 0 (induction start)	Aspirate CIHM-MED2, add pre-warmed CIHM-MED2B
Medium change frequency	Every 2–3 days (do not let medium become orange/yellow)
Differentiation duration	14–28 days (depending on mineralization level desired)
Culture conditions	37°C, 5% CO_2, humidified atmosphere

Staining and Validation

Stain	Detection	Expected result after 14–21 days
Alizarin Red S (pH 4.2)	Calcium deposits	Red-orange mineralized nodules
von Kossa	Phosphate deposits	Black/brown mineralized nodules

Stain	Detection	Expected result after 14–21 days
Alkaline Phosphatase (ALP)	Early osteoblast activity	Purple/blue staining (BCIP/NBT substrate)

Positive control: CIHM001 differentiated with CIHM-MED2B.
Negative control: CIHM001 maintained in CIHM-MED2 (no osteogenic supplements).

Performance Validation

Test	Acceptance Criteria	Result
Alizarin Red S staining (Day 21)	Visible red mineralization	Pass
ALP activity (Day 7–10)	>3-fold increase over negative control	Pass
Osteocalcin expression	Positive by immunocytochemistry or qPCR	Pass
RUNX2 upregulation	>5-fold by qPCR (Day 7 vs. Day 0)	Pass
Sterility	Negative for bacteria, fungi	Pass
Mycoplasma	Negative (PCR)	Pass
Endotoxin	< 0.5 EU/mL	Pass

Storage and Handling

- **Store at 2–8°C** in the dark. Do not freeze.
- **Protect from light** – Dexamethasone and ascorbic acid are light-sensitive. Keep in original opaque or amber bottle, or wrap with foil.
- **Pre-warm to 37°C** before use, but only once. Do not repeatedly heat-cool.
- **Use within 2 weeks after opening** – Ascorbic acid degrades rapidly at 37°C and in solution.
- **Discard if**:
    - Color changes to orange/yellow (pH drop from contamination or CO_2 loss)
    - Visible precipitate appears (may indicate degradation or calcium-phosphate crystallization)
    - Expiration date has passed

Quality Control

Each batch of CIHM-MED2B is tested for:

- Sterility (14-day test per USP <71>)
- Mycoplasma (PCR)
- Endotoxin (LAL assay)
- Functional differentiation capacity using CIHM001 (Alizarin Red S positivity by Day 21)
- Absence of cytotoxicity

Important Notes for Successful Differentiation

Consideration	Recommendation
Doxycycline carryover	Trace doxycycline in CIHM-MED1 can inhibit differentiation. Always use CIHM-MED2 for 48–72 hours before CIHM-MED2B.

Consideration	Recommendation
Cell density	Too sparse → poor cell-cell contact, reduced differentiation. Too confluent → multilayering, detachment. Optimal = 70–80% at start.
Medium changes	Gently aspirate and replace. Avoid disturbing adherent cells. Pre-warm medium to 37°C.
Mineralization timing	First visible Alizarin Red S staining appears around Day 14–18. Peak at Day 21–28.
Spontaneous mineralization	Rare in negative control (CIHM-MED2 only). If seen, verify doxycycline withdrawal and tetracycline-free serum.

Limitations and Warnings

Category	Statement
Intended use	For research use only. Not for human therapeutic, diagnostic, or veterinary use.
Dexamethasone	Corticosteroid. Avoid inhalation or skin contact. May be teratogenic – not for use by pregnant individuals without safety measures.
β-Glycerophosphate	May cause mild skin/eye irritation. Rinse thoroughly if exposed.

Category	Statement
Ascorbic acid	Degrades rapidly. Do not use medium if color has turned yellow-brown.
Disposal	Dispose as biohazardous waste per institutional guidelines.
Cell line compatibility	Validated only with CIHM001. Not tested with primary MSCs or other immortalized lines.

Ordering Information

Product	Catalog #	Volume
Osteogenic Induction Medium	CIHM-MED2B	500 mL
Doxycycline Withdrawal Medium	CIHM-MED2	500 mL
Complete Expansion Medium (Dox + Puro)	CIHM-MED1	500 mL
Alizarin Red S Staining Kit	CIHM-STAIN1	50 mL (ready-to-use)
Alkaline Phosphatase Stain (BCIP/NBT)	CIHM-STAIN2	100 mL

Related Products Recommended Workflow

Step	Product	Catalog #	Duration
Expansion	CIHM-MED1	CIHM-MED1	Until 70–80% confluent

Step	Product	Catalog #	Duration
Transgene silencing	CIHM-MED2	CIHM-MED2	48–72 hours
Osteogenic differentiation	CIHM-MED2B	CIHM-MED2B	14–28 days
Staining (optional)	Alizarin Red S Kit	CIHM-STAIN1	30 minutes

Storage and Stability Summary

Condition	Duration
Unopened, 2–8°C, dark	3 months
Opened, 2–8°C, dark	2 weeks
37°C water bath	<30 minutes (single use)
Repeated warming	Not recommended
Freezing (-20°C or -80°C)	Not allowed
Room temperature	< 2 hours (protected from light)