Data Sheet

Doxycycline Withdrawal Medium

Catalog Number: CIHM-MED2

Volume: 500 mL

Product Type: Cell culture medium for growth arrest and differentiation induction

Compatibility: CIHM001 (Conditionally Immortalized Human BM-MSCs)

Product Description

Doxycycline Withdrawal Medium (CIHM-MED2) is a complete, ready-to-use culture medium formulated specifically for the controlled growth arrest of Conditionally Immortalized Human Bone Marrow-Derived Mesenchymal Stem Cells (Catalog #CIHM001). These cells express SV40 Large T antigen (SV40 LT) under a TetON-inducible promoter. The presence of doxycycline drives proliferation; its withdrawal silences SV40 LT expression, reverting cells to a quiescent, primary-like state.

This medium contains **no doxycycline** and **no puromycin**, enabling the cessation of transgene expression and allowing differentiation studies or long-term quiescent culture. It retains all basal nutrients and growth supplements required for cell viability and lineage-specific differentiation.

Storage and Handling

Parameter	Instruction
Storage temperature	2°C to 8°C (refrigerate, do not freeze)
Light sensitivity	Protect from prolonged light exposure (no doxycycline, but medium contains light-sensitive vitamins)
Shelf life	6 months from the date of manufacture when stored properly
Pre-use preparation	No additional supplementation required; warm to 37°C before use
Color	Pink to reddish-pink (phenol red indicator)

Note: Do not freeze the medium; freezing causes precipitation of salts and loss of functionality.

Intended Use Instructions

For Growth Arrest (Transgene Silencing)

1. Expand CIHMSCs in complete expansion medium (CIHM-MED1) containing 2 µg/mL doxycycline and 1 µg/mL puromycin until 70–80% confluence.
2. Wash cells twice with PBS (warmed) to remove residual doxycycline and puromycin.
3. Replace medium with Doxycycline Withdrawal Medium (CIHM-MED2).
4. Culture for **48–72 hours** at 37°C, 5% CO_2 to achieve maximal SV40 LT silencing.

- *Verification*: Western blot for SV40 LT should show >90% reduction compared to control.

For Differentiation Experiments

After 48–72 hours in CIHM-MED2, cells are ready for exposure to lineage-specific differentiation media (not included):

- **Osteogenic differentiation:** Replace CIHM-MED2 with osteogenic induction medium
- **Adipogenic differentiation:** Replace with adipogenic induction medium
- **Chondrogenic differentiation:** Replace with chondrogenic induction medium

Differentiation media are available separately (contact technical support).

For Quiescent Maintenance (Optional)

Cells can be maintained in CIHM-MED2 for up to 10–14 days with medium changes every 2–3 days. Beyond 14 days, cell viability may decline, and spontaneous senescence may occur.

Expected Cell Behavior in CIHM-MED2

Parameter	Observed Outcome
SV40 LT expression	Silenced >90% within 48–72 hours
Proliferation	Arrested (no significant increase in cell number)
Viability	≥90% for up to 10 days

Parameter	Observed Outcome
Morphology	Remains spindle-shaped, fibroblast-like; no overt differentiation without lineage-specific cues
Differentiation potential	Preserved (osteogenic, adipogenic, chondrogenic) upon exposure to appropriate induction media
Reversible proliferation	Yes: Add doxycycline (2 μg/mL) to re-induce SV40 LT and resume proliferation

Quality Control

Test	Specification	Result
Sterility	No bacterial/fungal growth	Pass
Mycoplasma	Negative by PCR	Pass
Endotoxin	<0.5 EU/mL	Pass
pH	7.2 – 7.4	Pass
Osmolality	280 – 320 mOsm/kg	Pass
Doxycycline residue	Not detected (HPLC; detection limit 0.05 μg/mL)	Pass
Puromycin residue	Not detected (HPLC)	Pass
Functional test	Induces growth arrest in CIHM001 within 72h	Pass

Limitations and Warnings

- **For research use only.** Not for human therapeutic, diagnostic, or veterinary use.
- Do not mix with doxycycline-containing media; even trace doxycycline will reactivate SV40 LT expression.
- Use only **tetracycline-free FBS** in all media with these cells. Standard FBS contains tetracycline residues that cause leaky expression.
- Cells will not proliferate in this medium. For expansion, use CIHM-MED1.
- Prolonged culture (>14 days) without doxycycline may lead to irreversible senescence.
- Handle using aseptic technique; discard any medium showing turbidity, precipitate, or color change to yellow/orange (indicating pH drop from contamination).

Ordering Information

Product	Catalog #	Quantity
Doxycycline Withdrawal Medium	CIHM-MED2	500 mL
Conditionally Immortalized Human BM-MSCs	CIHM001	500,000 cells/vial
Complete Expansion Medium (with doxycycline + puromycin)	CIHM-MED1	500 mL
Puromycin (1 mg/mL)	CIHM-SEL1	1 mL

Product	Catalog #	Quantity
Doxycycline Solution (2 mg/mL)	CIHM-IND1	1 mL
Osteogenic Differentiation Medium	CIHM-DIFF1	100 mL
Adipogenic Differentiation Medium	CIHM-DIFF2	100 mL
Chondrogenic Differentiation Medium (pellet format)	CIHM-DIFF3	50 mL

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9. Troubleshooting Guide

Problem	Possible Cause	Solution
Cells continue to proliferate	Residual doxycycline carryover	Wash cells more thoroughly (3x PBS); ensure FBS is tetracycline-free
Cell death within 3–5 days	Withdrawal of puromycin not tolerated?	CIHM001 should survive without puro for >10 days. Check mycoplasma contamination.
SV40 LT still detected after 72h	Incomplete silencing	Extend withdrawal to 96h; verify no doxycycline in incubator CO_2 lines (rare)
Differentiation fails	Withdrawal period too short	Ensure ≥48h doxycycline-free before differentiation induction