Data Sheet

Astrocyte Thawing Medium

Catalog Number: MEDAST-T1
Volume: 50 mL
Product Format: Ready-to-use, sterile, serum-containing recovery medium
Lot Number: Provided on bottle label and Certificate of Analysis

PRODUCT DESCRIPTION

Astrocyte Thawing Medium (Catalog # MEDAST-T1) is a complete, sterile, optimized formulation specifically designed to maximize post-thaw viability, attachment, and functional recovery of cryopreserved human iPSC-derived astrocytes (e.g., Cat # IPSCDA001) and primary astrocyte cultures. The medium contains a proprietary combination of serum, antioxidants, and a cell-protective reagent that minimizes cryoprotectant (DMSO) toxicity, reduces osmotic shock, and prevents membrane damage during the thawing process.

Key Features:

- **High viability recovery:** ≥85% viable cells post-thaw (validated with Cat # IPSCDA001)
- **Serum-containing formulation** (10% FBS) – enhances membrane stabilization
- **Contains DNase I** – reduces clumping from dead cell DNA release
- **Contains antioxidant cocktail** – reduces post-thaw oxidative stress
- **Ready-to-use** – no additional supplementation required
- **Single-use format** – 50 mL sufficient for thawing up to 10 vials (5 mL/vial)

Note: Does **not** contain growth factors (FGF-2, EGF). For long-term culture, replace with Astrocyte Maintenance Medium (Cat # MEDAST-M1) 24 hours post-thaw.

STORAGE & HANDLING

Parameter	Condition
Storage temperature	2°C to 8°C (DO NOT FREEZE)
Shelf life (unopened)	3 months from date of manufacture
Shelf life (opened)	2 weeks when stored aseptically at 2–8°C
Protection from light	Not required (no light-sensitive components)
Pre-warming before use	37°C water bath for 10–15 minutes (DO NOT exceed 37°C)
Color indicator	Rosy pink (pH 7.2–7.4) → Yellow indicates pH drop (contamination or CO_2 imbalance)
Aliquot recommendation	Do not refreeze; discard unused portion after 2 weeks

THAWING PROTOCOL (For Cat # IPSCDA001)

Preparation

1. **Pre-coat culture vessels** (ideally day before or at least 2 hours before thawing):
    - Smooth Coat Solution overnight at 37°C, rinse twice with PBS,
    - **Pre-warm** Astrocyte Thawing Medium to 37°C (water bath or incubator).

Thawing Procedure (Do Not Centrifuge Immediately)

Step	Action
1	Remove vial of astrocytes (Cat # IPSCDA001) from liquid nitrogen.
2	Loosen cap ¼ turn to relieve pressure, then retighten.
3	Thaw in 37°C water bath with gentle agitation until **small ice crystal remains** (~90–120 seconds). Do not over-thaw.
4	Decontaminate vial with 70% ethanol.
5	**Immediately** transfer cells dropwise into 10 mL **pre-warmed** Astrocyte Thawing Medium in a 15 mL conical tube.
6	**Centrifuge at 300×g for 5–7 minutes at room temperature** (not 4°C – cold reduces viability).
7	Aspirate supernatant (contains DMSO).
8	Gently resuspend pellet in **1–2 mL pre-warmed Astrocyte Thawing Medium**.
9	Count cells (trypan blue exclusion). Expected viability: ≥85%.
10	Dilute to the desired seeding density in **Astrocyte Thawing Medium** (not maintenance medium yet).
11	Plate cells onto a coated vessel.
12	**Critical step:** 4–6 hours post-plating, replace Thawing Medium with **Astrocyte Maintenance Medium** (Cat # MEDAST-M1) to remove serum and DNase I.

Why replace at 4–6 hours? Longer exposure to serum promotes astrocyte reactivity and fibroblast overgrowth. Short exposure (4–6h) is sufficient for attachment but minimizes phenotypic drift.

VALIDATION DATA (Using Cat # IPSCDA001)

Parameter	Result with MEDAST-T1	Result with standard DMEM/F12 + 10% FBS
Viability (immediate post-thaw)	88 ± 3%	72 ± 6%
Attachment efficiency (24h)	92 ± 4%	68 ± 8%
Clumping (visible aggregates)	Minimal (<5% of cells)	Moderate–high (15–25% of cells)
GFAP expression (day 5)	91 ± 4% positive	73 ± 9% positive
IL-6 response to TNFα/IL-1β (day 7)	14-fold increase	6-fold increase

Data represent mean ± SD from 3 independent lots of iPSC-astrocytes, n=6 replicates per condition.

QUALITY CONTROL SPECIFICATIONS

Test Parameter	Specification	Result (representative lot)
Appearance	Clear, pink solution	Clear, pink solution ✅
pH (at 25°C)	7.2 – 7.4	7.3 ✅
Osmolality (mOsm/kg)	320 – 360	338 ✅
Sterility (14-day culture)	No bacterial/fungal growth	Negative ✅
Mycoplasma (PCR)	Negative	Negative ✅
Endotoxin (LAL assay)	<1.0 EU/mL	0.4 EU/mL ✅
DNase I activity	Functional (degrades DNA gel)	Positive (30 min, 37°C) ✅
Cell performance test*	≥85% viability + ≥85% GFAP+ at day 5	88% viability, 91% GFAP+ ✅

*Performance tested using Human iPSC-Derived Astrocytes (Cat # IPSCDA001) thawed per protocol, plated on a Smooth Coat Solution Coated Plate.

COMPATIBLE CELL TYPES

Cell Type	Catalog #	Recommended?
Human iPSC-Derived Astrocytes	IPSCDA001	✅ Optimized
Human iPSC-Derived Astrocytes (P2)	IPSCDA002	✅ Yes
Primary human fetal astrocytes	Various	✅ Yes

Cell Type	Catalog #	Recommended?
Primary rat/mouse cortical astrocytes	N/A	✅ Yes
Human iPSC-Derived NPCs (for thawing)	IPSCN001	❌ No (use NPC Thawing Medium, Cat # MEDNPC-T1)
Human iPSC-Derived Neurons	IPSCNEU001	❌ No (use Neuron Thawing Medium)

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Low viability (<70%)	Thawed too slowly; DMSO toxicity	Thaw rapidly (<2 min); use pre-warmed medium.
Excessive clumping	DNA release from dead cells	Medium contains DNase I; ensure 50 U/mL is active (store at 2-8°C).
Poor attachment (<50% after 24h)	Coating inadequate or cells seeded in maintenance medium	Use recommended coating; **do not** seed in serum-free medium.

Problem	Possible Cause	Solution
Floating cells after 4-6h	Normal (dead cells detach)	Gently wash with warm PBS before adding maintenance medium.
Cells look rounded at 24h	Serum reaction or endotoxin	Check endotoxin (<1 EU/mL); replace medium at 4-6h exactly.
Yellow medium before use	CO_2 exposure during storage	Discard; do not use if pH is orange/yellow.

APPLICATIONS FOR RECOVERED ASTROCYTES

After thawing, astrocytes are suitable for:

Application	Time post-thaw
Immunocytochemistry (GFAP, S100β, AQP4)	Day 5–7
Glutamate uptake assays	Day 5–7
Cytokine stimulation (TNFα, IL-1β, LPS)	Day 6–8

Application	Time post-thaw
Neuron-astrocyte co-culture	Plate astrocytes day 0, add neurons day 5–7
Calcium imaging (spontaneous or ATP-evoked)	Day 6–10
Phagocytosis (myelin, synaptosomes)	Day 7–10
RNA extraction / qPCR	Day 5–10

PRECAUTIONS & LIMITATIONS

- **For research use only.** Not for clinical, diagnostic, or therapeutic use.
- **Do not use if medium is turbid, contains precipitate, or is yellow/orange.**
- **Do not freeze** – serum components will denature.
- **Do not use for neurons or NPCs** – serum in this medium induces neuronal differentiation and NPC adhesion changes.
- **Replace with maintenance medium within 4–6 hours** – prolonged serum exposure causes astrocyte reactivity and proliferation of non-astrocyte contaminants.
- **Use within 2 weeks of opening** – discard any unused medium after 14 days.

ORDERING INFORMATION & RELATED PRODUCTS

Product	Catalog Number	Size
Astrocyte Thawing Medium	MEDAST-T1	50 mL
Astrocyte Maintenance Medium (serum-free)	MEDAST-M1	500 mL
Astrocyte Maintenance Medium (low serum: 1% FBS)	MEDAST-M1-S	500 mL
Human iPSC-Derived Astrocytes (P1)	IPSCDA001	500,000 cells
Human iPSC-Derived Astrocytes (P2 expanded)	IPSCDA002	1 million cells
Coating Kit	ECM-AST1	1 kit
Coating Kit (for NPCs & astrocytes)	ECM-NPC1	1 kit
DNase I (animal-free, 2500 U)	ENZ-DN1	1 vial
Astrocyte Functional Assay Kit (IL-6 ELISA + Glutamate uptake)	ASY-AST1	96 tests

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