Senso-DMmax Quick Guide

Catalog # 1011-B005
Version 1.1
Last Updated August 8, 2025

ANATOMIC
Not Your Average Neuron™

Technical Support | Email: support@anatomic.com | **Phone:** 612-208-6735

Handling and Storage

Upon receipt, immediately transfer components to the proper storage temp.

Component	Catalog #	Storage	Amount
Senso-DMmaxSupplements 1 – 7	1011	-20°C	1.5 mL vial x 7
Basecamp **Differentiation** Basal	B005	-20°C	121.5 mL bottle

Accompanying Reagents (Sold Separately)

Component	Catalog #	Storage	Amount
iMatrix 511-SILK	M511S	4°C	6 x 350 uL vial
Senso-MM (Maturation Media)	1030	-20°C	120 mL bottle

Other Reagents Needed

Component	Vendor	Catalog #
6-Well Polystyrene Plates	Fischer Scientific	FB012927
DMEM/F12	Gibco™	11330057
dPBS without Calcium/Magnesium	Corning	21-031-CV
Versene EDTA Solution	Gibco	15040066
TeSR E8 Medium (or equivalent)	StemCell	5990
Y-27632 (Dihydrochloride)	StemCell	72304
Chroman-1	Medchem Express	HY-15392
Accumax	Innovative Cell Tech	AM105
Cryostor CS10	BioLife Solution	210102

Successful differentiations are density-dependent, so an initial optimization is important when testing new hiPSC lines. We recommend using your first kit to optimize seeding densities across a full 6-well plate. Aim for a sparse distribution that allows visible neural crest formation by Day 4 and gradually reaches higher confluency toward the endpoint (see page 2 for representative pictures of the differentiation). While neurons can still form at lower densities, yields will be limited. The optimal density with Senso-DM MAX mirrors the optimal density with Senso-DM, and correlates with growth rate of the hiPSC line (slower hiPSC growth will require higher seeding densities). Once optimized, the protocol scales proportionally to T75 flasks without further modification.

hPSC Maintenance for Differentiation

1. Differentiation was optimized for E8/vitronectin based cultures
2. Grow cultures on a 4-day passage cycle in 6-well plates
3. Feed 2 mL, 2.5 mL, 3 mL, and 4 mL/well on days 0, 1, 2, and 3 respectively
4. By the 4th day cultures should be 80% confluent and ready for passage

Preparing Cell Culture Surface

1. Dilute 60 uL iMatrix 511-SILK 1:100 into 6 mL dPBS -/-
2. Add 1 mL diluted iMatrix 511-SILK per well of a 6-well plate
3. Incubate three hours at 37°C or overnight at 4°C (wrapped in parafilm to prevent evaporation).

Do not let vessels dry out during incubation or storage

Formulating E8 with Rock Inhibitor for Single-Cell Seeding

1. Combine complete E8 medium with 10 uM Y-27632
2. Alternatively, combine complete E8 medium with 50 nM Chroman-1

Single-Cell Seeding of hPSCs for Differentiation

1. Rinse well twice with 1 mL per wash Versene EDTA
2. Incubate cultures in 1 mL Versene EDTA for 20 minutes at 37°C
3. Triturate suspension harshly with P1000 to ensure single-cell suspension
4. Dilute with 4 mL DMEM/F12 and sample cells for counting
5. Centrifuge culture 300 x G for 4 minutes
6. Pour off supernatant and flick pellet vigorously to homogenize
7. Resuspend pellet to 1 million cells per mL in DMEM/F12
8. Manually pipet the iMatrix 511-SILK solution from the coated 6-wells
9. Add 2 mL per well each of the E8 + Rock Inhibitor to the coated 6-wells
10. Inoculate the wells directly with the volumes indicated in Table 1
11. Shake plate inside incubator back and forth to evenly distribute cells

Table 1: Volumes for Seeding Single-Cell Suspension

Cells per cm^2	Volume (uL) per 6-well assuming 1M/mL
Well 1 – 7 K	67.2
Well 2 - 8 K	76.8
Well 3 - 9 K	86.4
Well 4 – 10 K	96
Well 5 - 11 K	105.6
Well 6 – 12 K	115.2

*assuming each 6-well is 9.6 cm^2

Differentiation with Senso-DM MAX Differentiation Kit

1. Thaw the Basecamp Differentiation Basal overnight
2. Thaw Senso-DM MAX 1 supplement at room temperature
3. Add **13.5 mL** of Differentiation Basal to a 50 mL conical tube
4. To this, add **1.5 mL** of Senso-DM MAX 1 supplement and mix well
5. Pipet off the E8 medium from the single-cell seeded 6-wells
6. To each well, add 2 mL combined Senso-DM MAX 1 Medium
7. Place culture back into incubator and wait approximately 24 hours
8. Repeat the same procedure daily in sequence, from Senso-DM MAX 2 through 7
9. On the day following the Senso-DM MAX 7 feed, cultures can be dissociated

Dissociation of Immature Sensory Neurons

1. Rinse cultures once with 1 mL Versene EDTA
2. Add 1 mL Accumax to each well of 6-well
3. Incubate cultures 37°C for 10 minutes

Dissociation studies show that after ~10 minutes in 100% Accumax, peripheral neurons begin detaching, leaving behind any CNS "rosettes." The number of rosettes present will vary depending on initial plating density and the extent of neural crest emergence. Since CNS rosettes are undesirable when generating peripheral neurons, we recommend not exceeding 15 minutes of Accumax treatment. Prolonged incubation can lead to peripheral neuron death. Incubate until the colony edges (where sensory neurons are located) begin to lift. For your first run, monitor cultures closely, checking every 5 minutes. Gently shake the plate to see if edges are peeling before proceeding.

4. Triturate cultures vigorously with P1000 pipet to create single-cells
5. Dilute each culture with 4 mL DMEM/F12 and sample for counting
6. Centrifuge each sample 300 xG for 4 minutes
7. Resuspend each sample to 1 to 3 million cells per mL in Cryostor CS10
8. Aliquot suspensions into cryovials
9. Cool to -80°C using appropriate slow freezing container
10. Transfer to liquid nitrogen storage the following day

Congratulations! You have just generated your own RealDRG hiPSC-derived sensory neurons! Neurons produced using Senso-DM MAX can be thawed and rapidly matured using Senso-MM as outlined in the RealDRG QuickGuide.

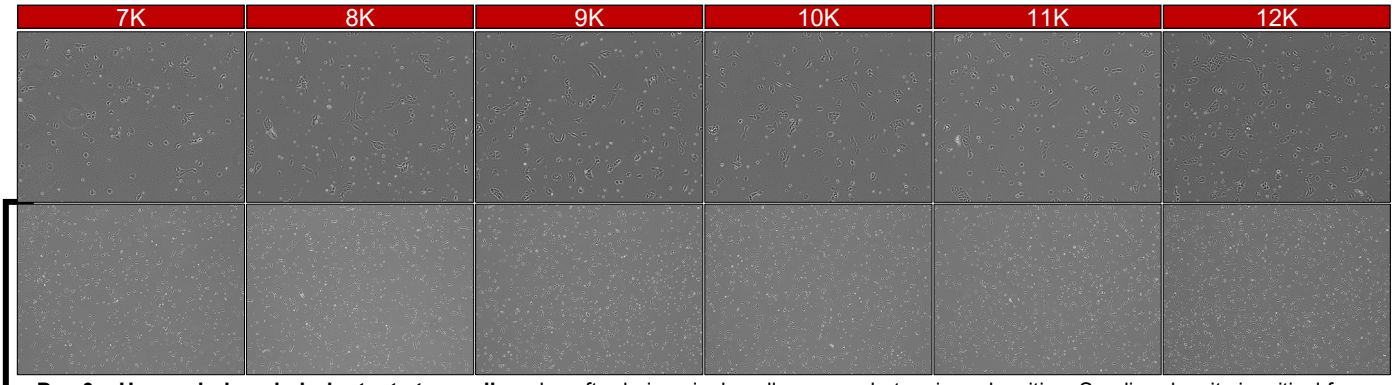
Senso-DMmax Quick Guide

Catalog # 1011-B005
Version 1.1
Last Updated August 8, 2025

ANATOMIC
Not Your Average Neuron™

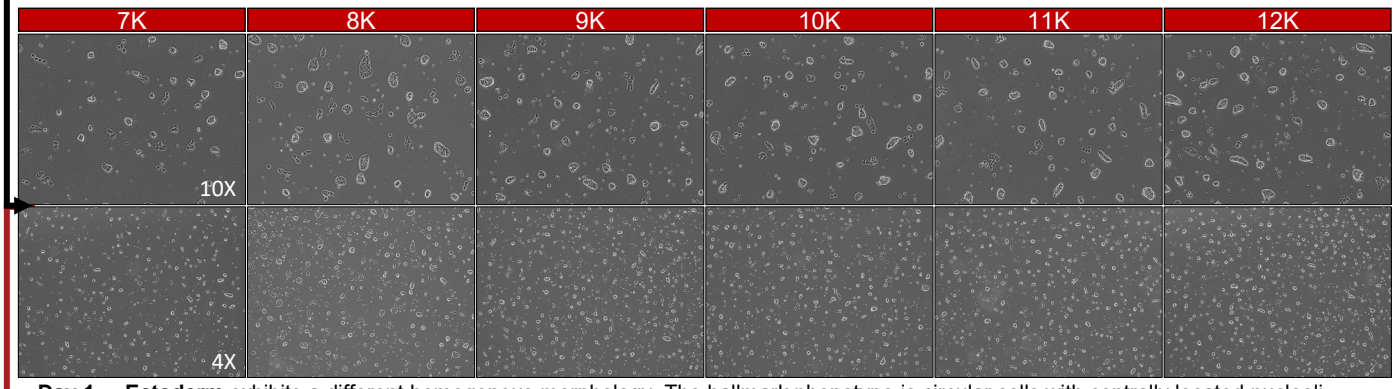
Technical Support | **Email:** support@anatomic.com | **Phone:** 612-208-6735

Senso-DM MAX 1



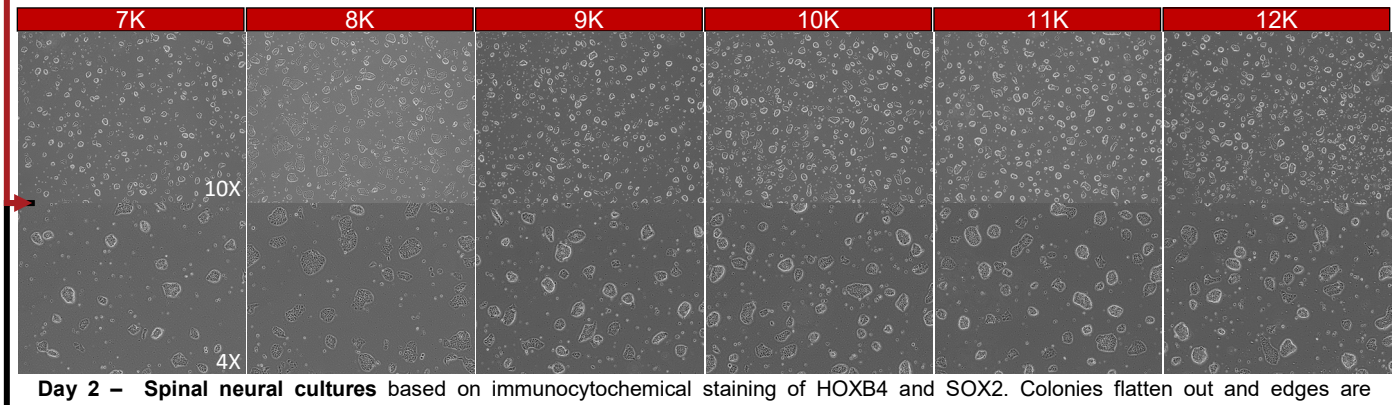
Day 0 – Human induced pluripotent stem cells a day after being single cell passaged at various densities. Seeding density is critical for a successful differentiation and needs to be optimized based on the innate growth of each hiPSC line.

Senso-DM MAX 2



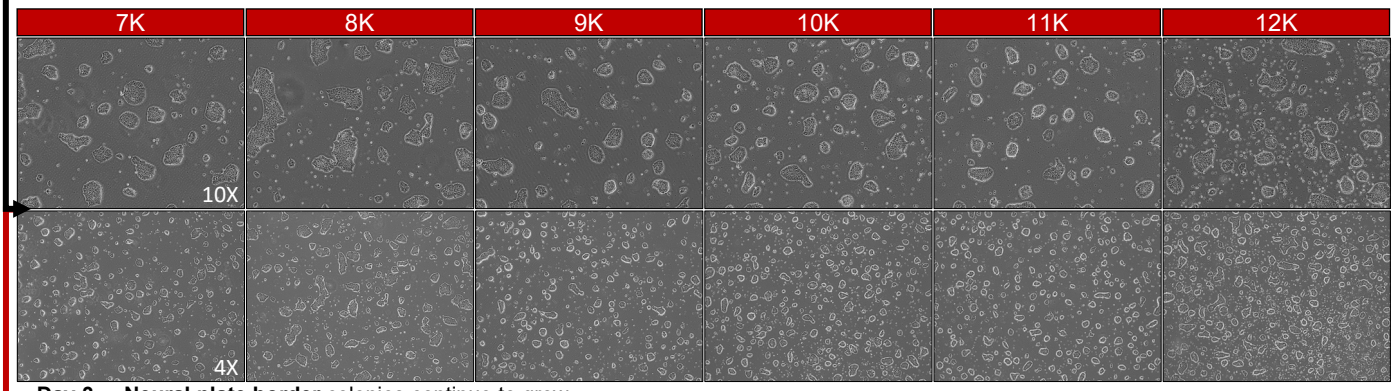
Day 1 – Ectoderm exhibits a different homogenous morphology. The hallmark phenotype is circular cells with centrally located nucleoli.

Senso-DM MAX 3



Day 2 – Spinal neural cultures based on immunocytochemical staining of HOXB4 and SOX2. Colonies flatten out and edges are smoother. The exhibit growth over the next couple of days.

Senso-DM MAX 4



Day 3 – Neural plate border colonies continue to grow.

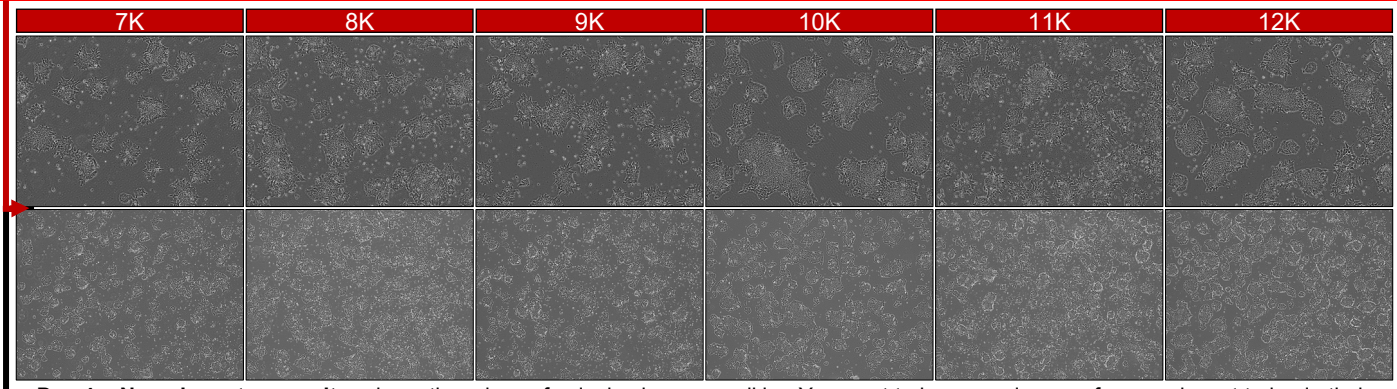
Senso-DMmax Quick Guide

Catalog # 1011-B005
Version 1.1
Last Updated August 8, 2025

ANATOMIC
Not Your Average Neuron™

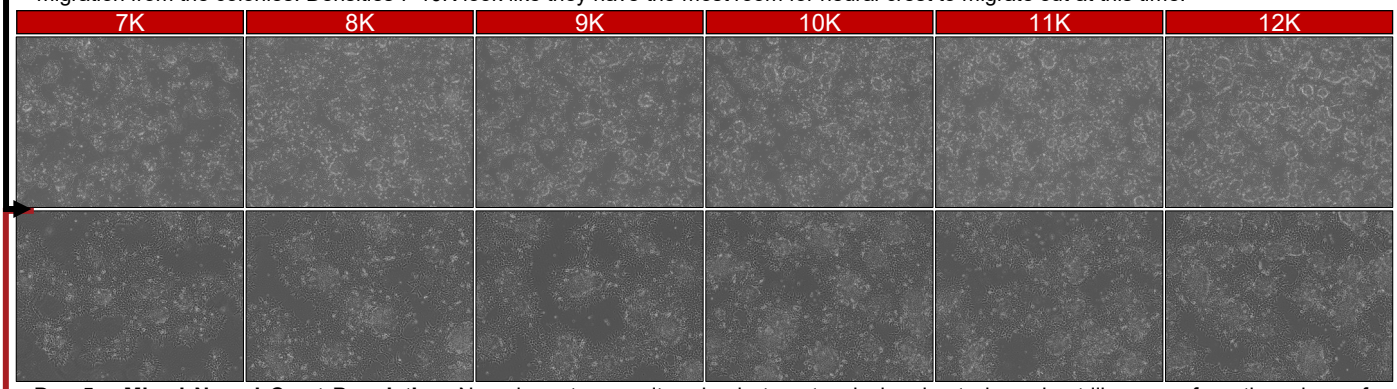
Technical Support | **Email:** support@anatomic.com | **Phone:** 612-208-6735

Senso-DM MAX 5



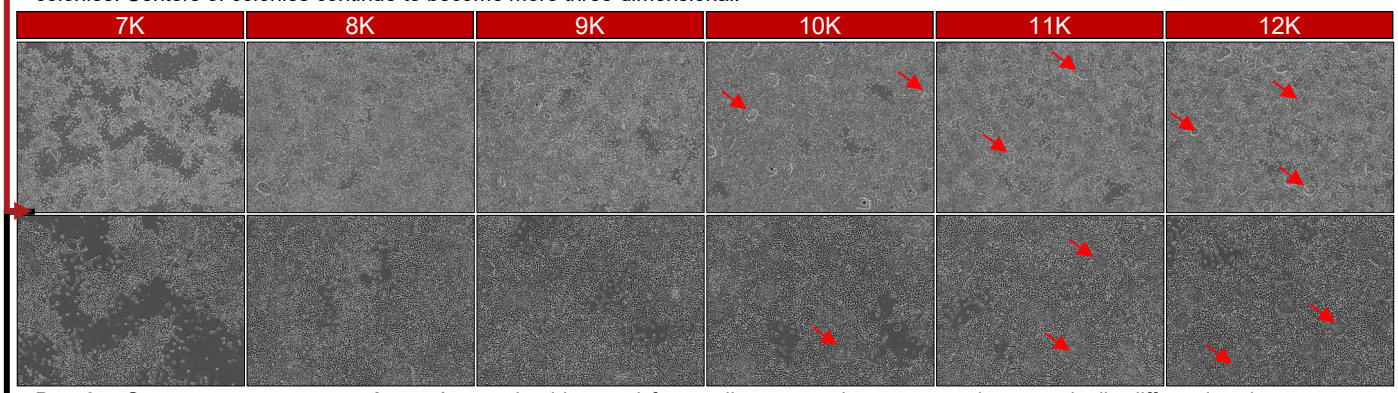
Day 4 – Neural crest progenitors have the edges of colonies become spikier. You want to have ample room for neural crest to begin their migration from the colonies. Densities 7-10K look like they have the most room for neural crest to migrate out at this time.

Senso-DM MAX 6

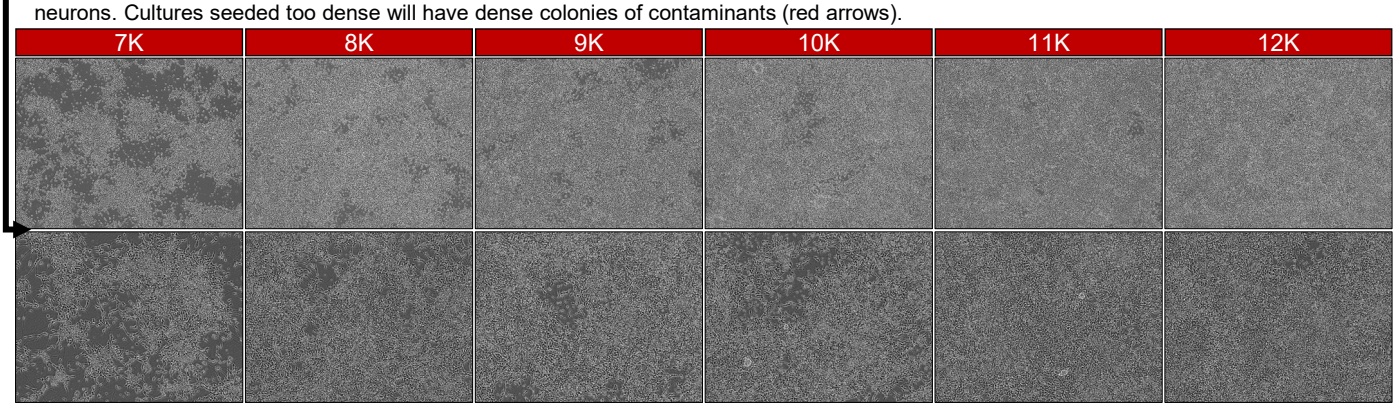


Day 5 – Mixed Neural Crest Population. Neural crest progenitors begin to extensively migrate in a sheet-like mass from the edges of colonies. Centers of colonies continue to become more three-dimensional.

Senso-DM MAX 7



Day 6 – Sensory neuron progenitors. Axons should extend from cells as neural crest progenitors terminally differentiate into sensory neurons. Cultures seeded too dense will have dense colonies of contaminants (red arrows).



Day 7 – Sensory Neurons. Axons of sensory neurons should continue to elongate and sensory neuron progenitors should continue to terminally differentiate. Cells can be banked at this point or continually be matured in culture. 7K seems to be too sparse. 8-9K is ideal.

Frequently Asked Questions

Storage

How long can the Senso-DM kits be stored under optimal conditions while maintaining functionality for differentiation?

- The differentiation kits and maturation media have a six-month expiration date from the time of manufacture. The expiration date is listed on each individual product label as well as the packing slip that the package came in. If needed, please reach out to customer service (support@anatomic.com) with the LOT#.

Can the Senso DM 1-7 supplements be aliquoted and used at different times without affecting efficacy?

- We have not tested re-aliquoting the differentiation kits. Most users differentiate multiple lines in parallel or use the full kit at once. We provide an additional kit for first-time users to optimize and experiment with multiple lines. Please share your intended use so we can provide the best recommendation.

Typical problems and solutions

Everything immediately died 24 hours after using Senso-DM1 MAX

- Ensure no more than 0.2 mL/cm2 of Senso-DM1 MAX was used on Day 0
- Incubation of iMatrix 511-SILK: Recommended incubation is three hours at 37°C or overnight at 4°C (wrapped in parafilm to prevent evaporation).
- Evaporation: If iMatrix 511-SILK dries out, it becomes less effective. Ensure only a thin film remains before adding media.
- TC plate expiration: Check expiration dates, as expired plates may affect results.

I don't notice a morphology change on day 1

- Look more closely (20x to 40X magnification)

Cells died mid-differentiation

- Manually extract culture media with serological pipet rather than vacuum aspirate to prevent cultures from drying out

My cultures didn't characterize to be highly positive for sensory neuronal markers

- Ensure use of Senso-DM in proper sequence: Senso-DM1->Senso-DM2->Senso-DM3->Senso-DM4->Senso-DM5->Senso-DM6->Senso-DM7
- Ensure cultures display neuronal morphology as undifferentiated neural crest will be flatter and stain negative for sensory neuron markers.

Freezing and Viability

How many vials can be obtained when freezing cells on Day 7 and what is the efficiency of freezing cells at this stage?

- Each kit yields approximately 10–12 million sensory neurons at the optimized seeding density. For cryopreservation, we typically target 1 million viable neurons per vial by freezing at 2 million cells/mL, anticipating a ~50% loss during thaw. Post-thaw recovery usually yields 1–1.5 million viable cells per vial, with viability around 80%. Note that cryopreservation is optional and cells may also be directly replated following differentiation without freezing.

Miscellaneous

How does Senso-DMmax (Cat#1011) differ from Senso DM (Cat#1010)?

- Optimized substrate - uses iMatrix 511-SILK (Cat# M511S) instead of Matrix 1, improving ease of use and increasing differentiation efficiency
- Larger kit size to allow initial users to test multiple seeding densities in their first run
- Shorter dissociation time for improved workflow efficiency

What hiPSC lines have customers used the differentiation kits on?

- Multiple customers have used their own hiPSC lines and from repositories including The Jackson Laboratory, Cedars Sinai, and CIRM. Based on Kalia et al., our Senso-DM kit works on lines that are difficult to differentiate with standard protocols like the Chambers protocol.

How long can RealDRG be cultured?

- We have cultured sensory neurons for up to 180 days post-thaw, with somas continuing to increase in size. Theoretically, the culture could have continued beyond this timeframe but even the scientists at Anatomic need to take a vacation.

Can you use antibiotics with the differentiation kit?

- We have not tried running a differentiation kit with antibiotics. You can culture RealDRG in 1X anti-anti or pen/strep.

At Anatomic, we are constantly improving our products to enhance the user experience and ensure reliable, consistent results. Our goal is to develop assays that not only simplify workflows but also work seamlessly the first time, every time.

Version Updates:

1.0 → 1.1

- Expanded FAQ to explain the differences between Senso-DM (Cat# 1010) and Senso-DMmax (Cat# 1011)
- Added more detail to the dissociation section
- Adjusted naming convention from Senso-DM MAX → Senso-DMmax (per marketing team preference)