ANATOMIC

hiPSC derived Sensory Neurons
User Guide

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1.0 Materials Required for Rapid Maturation of Sensory Neurons from hPSCs

The Chrono™ Sensory Neuron system is very simple to use and requires few materials and standard equipment to ensure success.

1.1 Product Contents and Storage

Component	Storage	Packaging	Total Volume	Stability
Sensory Neurons	Vapor Phase N_2	>1M viable cells/vial	1 mL	Until labeled expiry

1.2 Materials Required but not Included

Product Name	Supplier	Cat #	Use
iMatrix-511 SILK	Anatomic	M8003	Coating Buffer
Senso-MM	Anatomic	7008	Maturation Media
0.01% Poly-L-Ornithine	Millipore Sigma	A-004-C	Coating Buffer
dPBS w/o Calcium and Magnesium	Corning™	21031CV	Coating Buffer
Tissue-culture treated flasks, plates, or dishes	Your preferred supplier	-	Cell Culture
Glass coverslips	Your preferred supplier	-	Patch clamp electrophysiology
DMEM/F-12	Gibco™	11330057	Thaw
15 & 50 mL conicals	Your preferred supplier	-	Thaw
Hemocytometer	Your preferred supplier	-	Thaw
Trypan Blue	Your preferred supplier	-	Thaw

1.3 Required Equipment

- Biosafety cabinet certified for handling of biological materials
- Incubator (37°C, 5% CO2, 95% humidity)
- Pipette-aid with appropriate serological pipettes
- Water bath (37°C)
- Inverted microscope
- Freezer (-20°C)
- Refrigerator (2 - 8°C)

2.0 Preparation of Reagents and Media

2.1 Coating TC Plates and Glass Coverslips with Poly-L-Ornithine and iMatrix-511 SILK

2.1.1. Thaw 0.01% Poly-L-Ornithine solution at room temperature
2.1.2. Fully coat the cell culture surface with diluted Poly-L-Ornithine solution
2.1.3. Allow cell culture vessel to sit at room temperature overnight.
2.1.4. Aspirate the Poly-L-Ornithine solution the following day and rinse vessel with sterile water followed by coating with iMatrix-511 SILK
2.1.5. Dilute iMatrix-511 SILK 1:100 into dPBS (-/-)
2.1.6. Add 0.1 mL/cm^2 of iMatrix-511 SILK to tissue culture-treated vessels
2.1.7. Swirl the vessel to evenly spread the solution across the surface
2.1.8. Incubate the vessel overnight at 4°C or at least three hours at 37°C

!!!CRITICAL!!!: Do not let vessels dry out during storage and when aspirating iMatrix-511 SILK prior to cell seeding.

NOTE: Vessels can also be wrapped with parafilm and stored at 4°C overnight and up to two weeks before use. Smaller culture formats will be more prone to evaporation.

2.2 Preparation of Chrono™ Senso-MM

2.2.1. Thaw the appropriate amount of Chrono™ Senso-MM for the day at room temperature or overnight in the refrigerator
2.2.2 Aliquot remaining Chrono™ Senso-MM into appropriate amounts to store at -20°C.

!!!CRITICAL!!!: Chrono™ Senso-MM should not be freeze thawed more than twice.

Note: Chrono™ Senso-MM *DOES NOT* contain antibiotics or antifungal agents. Anatomic does not recommend the use of penicillin, streptomycin, amphotericin, and other antimicrobial agents. If proper aseptic techniques are adhered to, these antimicrobial agents should not be necessary.

3.0 Thawing and Plating Chrono™ Sensory Neurons

3.1 Based on your assay of interest, prepare coating of culture vessels as described above in 2.1 Coating Tissue Culture Plates or Glass Coverslips with Poly-L-Ornithine and iMatrix-511 SILK.
3.2 Thaw Chrono™ Senso-MM as described above 2.2 Preparation of Chrono™ Senso-MM.
3.3 Warm 10 mL DMEM/F12 to room temperature.
3.4 Remove the cryovial containing the frozen cells from liquid nitrogen storage and immediately place it into a 37°C water bath.

3.5 Quickly thaw the cells (< 1 minute) by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial. Do not submerge the vial.
3.6 Transfer the vial it into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.
3.7 Once thawed completely, gently transfer the cells into a sterile centrifuge tube.
3.8 Gently rinse the cryovial with 1 mL of warmed DMEM/F12 and transfer to the sterile centrifuge tube
3.9 Add 8 mL of warmed DMEM/F12 dropwise to the cell suspension in the centrifuge tube.
3.10 Centrifuge the cell suspension at approximately 300 × g for 4 minutes.
3.11 After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically aspirate the supernatant without disturbing the cell pellet.
3.12 Cap tube and gently flick pellet so that it smears in the conical. Gently resuspend the cell pellet in 2 mL of Chrono™ Senso-MM complete growth medium to create a smooth cell suspension.
3.13 Perform a cell count. Anatomic recommends a seeding density of 100K cells/cm^2 for fluorescence based assays and 40K cells/cm^2 for patch clamp electrophysiology.
3.14 Remove iMatrix-511 SILK from the culture vessel(s). Immediately add the appropriate volume of Chrono™ Senso-MM. Do not let the coating dry out during the process.
3.15 Transfer the sensory neurons into the appropriate culture vessel(s)
3.16 Place cultures into the incubator at 37°C, 5% CO2, and 95% humidity.
3.17 Gently rock the culture vessel(s) back and forth to ensure even plating of cells.

4.0 Maturation of Sensory Neurons using Chrono™ Senso-MM

The protocol for maturing Chrono™ Sensory Neurons is very straightforward. We recommend feeding with Chrono™ Senso-MM the day after plating and every other day with standard volumes. We typically feed Monday, Wednesday, and Friday leaving the weekend free.

4.1 Recommended Feed Volumes

Format	Growth Area (cm^2)	Media Volume (mL)
6-well	9.5	1.9-2.0
12-well	3.8	0.76-1.14
24-well	1.9	0.38-0.57
48-well	0.95	0.19-0.28
96-well	0.32	0.1-0.2
384-well	0.056	0.025-0.05

4.2 Feeding with Chrono™ Senso-MM

Prepare Chrono™ Senso-MM as indicated in Section 2.3: Preparation of Chrono™ Senso-MM.

4.2.1. Transfer hPSC culture vessel to biosafety cabinet

4.2.2. Manually extract culture medium from culture vessel with serological or micropipette

NOTE: Sensory Neurons are extremely susceptible to drying out, and vacuum aspiration should be strictly avoided.

4.2.3. Dispense the recommended amount of Chrono™ Senso-MM into the culture vessel

NOTE: Sensory Neurons may be loosely adherent following extended culture. It is recommended to carefully dispense media down wall of culture vessel to avoid direct pipetting only neuronal monolayer

4.2.4. Return culture to incubator

4.2.5. Incubate approximately 48 hours

5.0 Endpoint Analysis using Chrono™ Senso-MM

After 7 days of culture, sensory neurons cultured in Chrono™ Senso-MM show action potential firing and resting membrane potential around -50 mV. Microelectrode array studies show the appropriate drug responses to capsaicin and lidocaine. This data is supported by qPCR data that shows the expression of appropriate voltage-gated sodium ($Na_v1.7$, $Na_v1.8$, $Na_v1.9$) and calcium ion channels ($Ca_v2.2$) as well as the transient receptor potential ion channel (TrpV1) that play an important role in nociception. We suggest that you carry out your experiments with the neurons between days 7-10, but this is highly dependent on your assay of interest.

Please feel free to reach out to us to discuss your research goals with the Chrono™ Senso Products.