ANATOMIC

Chrono™ Senso-MM
Technical Manual

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1.0 Materials Required for Rapid Maturation of Sensory Neurons from hPSCs

Chrono™ Senso-MM is very simple to use and requires few materials and standard equipment to ensure success.

1.1 Product Contents and Storage

Component	Temperature	Packaging	Total Volume	Stability
Chrono™ Matrix 3	4°C	1 mL Tube	75 µL	Until labeled expiry
Senso-MM	-20°C	30 mL PET Bottle x 4	30 mL x 4	Until labeled expiry

1.2 Materials Required but not Included

Product Name	Supplier	Cat #	Use
dPBS without Calcium and Magnesium	Your preferred supplier	-	Coating Buffer

1.3 Required Equipment

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

2.0 Preparation of Reagents and Media

2.1 Coating with Chrono™ Matrix 3

2.1.1. Dilute Chrono Matrix 3 1:100 into dPBS without Calcium and Magnesium
2.1.2. Add 0.1 mL/cm^2 of Chrono™ Matrix 3 to tissue culture-treated vessels
2.1.3. Swirl the vessel to evenly spread the solution across the surface
2.1.4. 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 matrix 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

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

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

3.0 Maturation of Sensory Neurons using Chrono™ Senso-MM

The protocol for Chrono™ Senso-MM is very straightforward. We recommend feeding every other day with standard volumes.

3.1 Recommended Feed Volumes

Format	Growth Area (cm^2)	Media Volume (mL)
6-well	9.6	1.9-2.0
12-well	4.8	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

3.2 Feeding with Chrono™ Senso-MM

Prepare Chrono™ Senso-MM as indicated in Section 3.2: Preparation of Chrono™ Senso-MM.

3.2.1. Transfer Sensory Neuron culture vessel to biosafety cabinet

3.2.2. 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.

3.2.3. 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

3.2.4. Return culture to incubator

3.2.5. A feeding cadence of Monday, Wednesday, Friday is recommended.

4.0 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.

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