Axion Maestro Guide for RealMOTO™

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ANATOMIC
Not Your Average Neuron™

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Handling and Storage

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

Component (Items sold Separately)	Catalog #	Storage Temperature	Amount
RealMOTO™ Motor Neurons	3020	Liquid Nitrogen	1 vial
Moto-MM (Maturation Media)	3030		
Moto-MM Basal		4°C	115 mL bottle
Moto-MM Supplement		-20°C	5 mL bottle
iMatrix-511 SILK	M511S	4°C	6 x 350 uL tube

Other Reagents Needed

Component	Vendor	Catalog #
CytoView MEA 24-White	Axion Biosystems	M384-tMEA-24W-5
0.01% Poly-L-Ornithine Solution	Sigma-Aldrich	A-004-C
dPBS (-/-)	Various	-
Cell Culture Grade Water	Various	-
DMEM/F12	Gibco™	11330057
Antibiotic-Antimycotic (100X)	Gibco™	15240062
BrainPhys™ Neuronal Medium	STEMCELL Tech	05790

Preparing CytoView MEA Surface

1. Add ~7mL cell culture grade water with 1X dilution of anti-anti to the outer rim of the plate to keep a humid environment
2. Coat electrodes with 35uL per well 0.01% PLO overnight at room temperature -the coating should cover the see-through portion of the plate with the electrodes mini well inside the larger well)
3. Wash off PLO 4x with 500 uL cell culture grade water - ensure the PLO and water is completely removed between washes
4. Add 35uL per well iMatrix-511 SILK as a secondary coating
5. Incubate at 37°C for 3 hours

Do not let vessels dry out during storage and when aspirating iMatrix-511 SILK prior to cell seeding as this will deactivate the coating

Preparing Maturation Medium

1. Thaw Moto-MM supplement at room temperature
2. Add this 5 mL supplement to the 115 mL Basecamp Basal bottle
3. **Store Moto-MM at 4°C for only up to 1 week.**
4. For long term storage, aliquot remaining Chrono™ Moto-MM into appropriate amounts to store at -20°C.
5. Equilibrate Moto-MM to room temperature before use .

Do not use a 37°C water bath to thaw media

Thawing the Cells

1. Warm 10 mL DMEM/F12 to room temperature.
2. Remove the cryovial from liquid nitrogen storage and immediately place it into a 37°C water bath.
3. 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.
4. Transfer the vial it into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.

Thawing the Cells (continued)

5. Once thawed completely, gently transfer the cells into a sterile centrifuge tube.
6. Gently rinse the cryovial with 1 mL of warmed DMEM/F12 and transfer to the sterile centrifuge tube
7. Add 8 mL of warmed DMEM/F12 dropwise to the cell suspension in the centrifuge tube. The initial drops should be slow to prevent osmotic shock and low viability. This step should take ~1 minute to complete.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Plating out RealMOTO™

Anatomic recommends a general seeding density of 80K neurons per well distributed as a monolayer across the whole surface area of the well (roughly 400K neurons/cm2. Higher seeding densities could result in clustering, peeling along the edge of the well, and higher baseline activity. This is highly dependent on your donor lines and will likely need to be optimized. Pictures of representative seeding densities are on the following page. Please contact Technical Support for assay-specific seeding recommendations.

1. After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically aspirate the supernatant without disturbing the cell pellet.
2. Cap tube and gently flick pellet so that it smears in the conical. Gently resuspend the cell pellet in Moto-MM complete growth medium to create a smooth cell suspension.
3. Perform a viable cell count. Resuspend cells to the appropriate seeding density based on donor line. **For 80K neurons per well, resuspend to 2M/mL and seed with 45.8 uL.**
4. Remove iMatrix-511 SILK from the culture vessel(s) and immediately seed with the cells.
5. Place cultures into the incubator at 37°C, 5% CO_2, and 95% humidity for 15 minutes to allow cells to settle.
6. Gently fill the well with 500 uL media (1X anti-anti).

Maintenance of Cells

Avoid dislodging the RealMOTO™ motor neurons by dispensing medium GENTLY as the cells can easily detach during culture handling.

1. Perform a **150%** media exchange with Moto-MM the day after plating. (Remove 500 uL per well and add 750 uL)
2. Perform a **2/3** media exchange with Moto-MM every 2 days (ie. Monday, Wednesday, and Friday).
3. Refill the outer rim of the plate with cell culture water (with 1X anti-anti) when the water levels run low (every other feed).
4. Culture the cells at 37°C, 5% CO_2, and 95% humidity.

Baseline Recordings (Day Before)

1. On the day before a recording, gently remove Moto-MM from the majority of the well, but leave media on the small area where the cells and electrodes are.
2. Add 495 uL BrainPhys™ Neuronal Medium to the wells. This helps increase network activity including burst spiking.

Baseline Recordings (Day Of)

Perform readings at least 24 hours after a media exchange

1. Set temperature to 37°C and CO_2 to 5% and allow instrument chamber to equilibrate
2. Add plate and do a baseline recording for 10 minutes
3. Gently remove BrainPhys™ Neuronal Medium from the majority of the well and replace with Moto-MM for future recordings.

Baseline Studies (Day Before)

1. On the day before a recording, gently remove Moto-MM from the majority of the well, but leave media on the small area where the cells and electrodes are.
2. Add 495 uL BrainPhys™ Neuronal Medium to the wells. This helps increase network activity including burst spiking.

Drug Studies (Day Of)

Perform readings at least 24 hours after a media exchange

1. Set temperature to 37°C and CO_2 to 5% and allow instrument chamber to equilibrate
2. Prepare drugs to 100X working dose concentration with at most 1% DMSO/Water/EtOH.
3. Include a vehicle control in your experiments to account for addition artifacts
4. Run a 5 minute baseline with lid off the plate in the Axion machine
5. Manually open the Axion Maestro system and add 5 uL working drug to desired wells (100X dilution)
6. Record what time drugs are dosed.
7. Allow Axion to record for an additional 5 minutes to 24 hours based on your drug's mechanism of action.