RealMOTO™ Motor Neurons Quick Guide

ANATOMIC

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 #
TC Polystyrene Plates	Various	-
Poly-L-Ornithine Solution (0.01%)	Sigma-Aldrich	A-004-C
dPBS (-/-)	Various	-
DMEM/F12	Gibco™	11330057

Preparing Cell Culture Surface

For most applications, use cell culture vessels or glass coverslips pre-coated with Poly-L-Ornithine and iMatrix-511 SILK. Plate surface areas and volumes vary based on vendors and assay of interest. The following are general recommendations. Please contact Technical Support for assay-specific cell culture surface recommendations.

Culture Vessel	Surface Area (cm^2)	iMatrix 511-SILK Dilution	Coating Volumes
6-well Plate	9.6	1:100	1 mL
12-well Plate	3.5	1:100	500 uL
24-well Plate	1.9	1:100	250 uL
96-well Plate	0.32	1:50	75 uL
384-well Plate	0.1	1:25	25 uL

1. Thaw Poly-L-Ornithine solution at room temperature.
2. Fully coat the cell culture surface with diluted Poly-L-Ornithine solution.
3. Parafilm and allow cell culture vessel to sit at room temperature overnight.
4. Aspirate the Poly-L-Ornithine solution the following day and rinse vessel 2X with sterile water.
5. Dilute iMatrix-511 SILK based on plate format into dPBS (-/-)
6. Add iMatrix-511 SILK to tissue culture-treated vessels.
7. Incubate the vessel overnight at 4°C or at least two hours at 37°C.

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

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.
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.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Plating out RealMOTO™

Anatomic recommends a general seeding density of 10K-15K cells/cm2, but this is highly dependent on your assay of interest and time points to test. Motor neurons clump at higher seeding densities which is controllable with the recommended sending density. Pictures of representative seeding densities are on the following page. Please contact Technical Support for assay-specific seeding recommendations.

Culture Vessel	Seeding Volume	Seeding Density ($cells/cm^2$)	Cells/Well
6-well Plate	2 mL	10K-15K	96K-144K
12-well Plate	1 mL	10K-15K	35K-52.5K
24-well Plate	500 uL	10K-15K	19K-28.5K
96-well Plate	100 uL	10K-15K	3.2K-4.8K
384-well Plate	50 uL	10K-20K	1-2K

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 2 mL of 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 assay of interest.
4. Remove iMatrix-511 SILK from the culture vessel(s). Immediately add the appropriate volume of Moto-MM. Do not let the coating dry out during the process.
5. Transfer the motor neurons into the appropriate culture vessel(s)
6. Place cultures into the incubator at 37°C, 5% CO2, and 95% humidity.
7. Gently rock the culture vessel(s) back and forth to ensure even plating of cells.

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.
2. Perform a **2/3** media exchange with Moto-MM every 2 days (ie. Monday, Wednesday, and Friday). Cultures can be fed two-days in a row to align with this MWF schedule.
3. Culture the cells at 37°C, 5% CO2, and 95% humidity.

Contacting Technical Support

Email: support@anatomic.com

Phone: 612-208-6735

Research Use Only

Version 1.0

RealMOTO™ Motor Neurons Immunocytochemistry Quick Guide

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Not Your Average Neuron

Table 2: Immunopanel for ICC Characterization

Component	Dilution	Vendor	Catalog #
HB9 (MNX1) antibody	1:25	DSHB	81.5C10
Islet-1 antibody	1:250	R&D Systems	AF1837
CHX10	1:250	Exalpha	X1179P
NKX2.2	1:125	DSHB	74.5A5
OLIG2	1:500	Millipore	AB9610
FOXA2	1:500	R&D Systems	AF2400
Beta III Tubulin	1:250	Millipore	MAB1637
Choline Acetyltransferase	1:100	Millipore	AB144P
488 Donkey anti-Mouse 2°	1:1000	Invitrogen	A-21202
555 Donkey anti-sheep 2°	1:1000	Invitrogen	A21436
555 Donkey anti-rabbit 2°	1:1000	Invitrogen	A31572
555 Donkey anti-goat 2°	1:1000	Invitrogen	A21432

Other Immunocytochemical Reagents

Component	Vendor	Catalog #
dPBS (calcium, magnesium)	Life Technologies	14040117
Formalin 1:10 dilution (buffered)	Fisher Scientific	23-305-510
Triton X-100	Sigma-Aldrich	P7949-500ML
Tween 20	Sigma-Aldrich	P7949-500ML
Bovine serum albumin	Sigma-Aldrich	A-9430-25G
Water for Cell Culture	Life Technologies	A1287303
DAPI Dilactate	Life Technologies	D3571

Permeabilization Buffer:
0.4% (v/v) Triton X-100 in dPBS +/+
Blocking Buffer:
0.2% (v/v) Tween 20, 2% bovine serum albumin (w/v) in dPBS+/+
DAPI Counterstain Stock solution:
Dilute 10 mg DAPI into 2 mL water (1000x concentrate)
Staining Dilutions
Perform counterstain and antibody dilutions in Blocking Buffer

All liquid exchanges should be performed as gently as possible to avoid disruption of cultures. The goal is to always leave ~50 uL of liquid remaining in the well so pipet tips do not disrupt the axonal network.

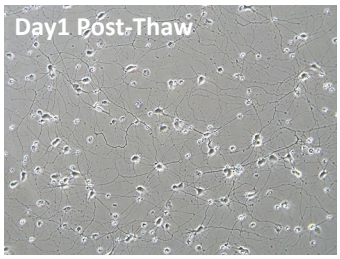
Fixation
1. The assumed starting amount in a 24-well is 250 uL.
2. Add 250 uL Formalin to culture medium
3. Incubate room temperature 10 minutes
4. Aspirate 400 uL solution

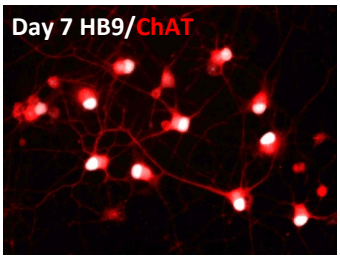
Permeabilization:
1. Add 250 uL Permeabilization Buffer
2. Incubate room temperature 10 minutes
3. Remove 250 uL Permeabilization Buffer

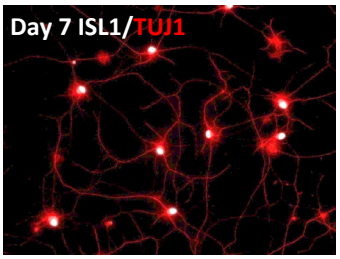
Intracellular Primary, Secondary, Counterstain
1. Add 150 uL diluted primary antibodies in blocking buffer
2. Parafilm-wrap edges of tissue culture vessel
3. Incubate overnight 4C
4. Aspirate 150 uL diluted primary antibodies
5. Add 150 uL Blocking Buffer
6. Aspirate 150 uL Blocking Buffer
7. Add 150 uL diluted secondary antibodies and DAPI in blocking buffer
8. Foil-wrap tissue culture vessel
9. Incubate room temperature in darkness 30 minutes
10. Aspirate 150 uL diluted secondary antibodies and DAPI
11. Add 250 uL dPBS
12. Aspirate 250 uL dPBS
13. Add 250 uL dPBS
14. Aspirate 250 uL dPBS
15. Add 500 uL dPBS
16. Parafilm-wrap edges of tissue culture vessel
17. Store 4C in darkness for downstream imaging

Perform additional dPBS washes as necessary if background is still high.

Figure 1: Typical Results from RealMOTO







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