RealMOTO™ & RealSCP™ Co-culture Quick Guide

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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 N_2	1 vial
Moto-MM Kit (Maturation Media)	3030		
Basecamp Maturation Basal		4°C	115 mL bottle
Moto-MM Supplement		-20°C	5 mL bottle
RealSCP™ Schwann cell Precursors	4020	Liquid N_2	
MotoSCP Co-culture Kit	3032		
Basecamp Maturation Basal		4°C	115 mL bottle
MotoSCP Co-Culture 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
Chroman-1	Medchem Express	HY-15392

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 Moto-MM Kit (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 RealMOTO™

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 50 mL 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 with continuous agitation/swirling. Transfer this culture to 15 mL tube to pellet.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Seeding RealMOTO™

Anatomic recommends a general seeding density of 20K cells/cm2 for co-cultures, 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 seeding 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	20K	192K
12-well Plate	1 mL	20K	70K
24-well Plate	500 uL	20K	38K
96-well Plate	100 uL	20K	6.4K
384-well Plate	50 uL	20K	2K

1. After the centrifugation, check the clarity of supernatant and visibility of a compact pellet. Aseptically withdraw the supernatant with a serological pipet 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.

Contacting Technical Support

Email: support@anatomic.com

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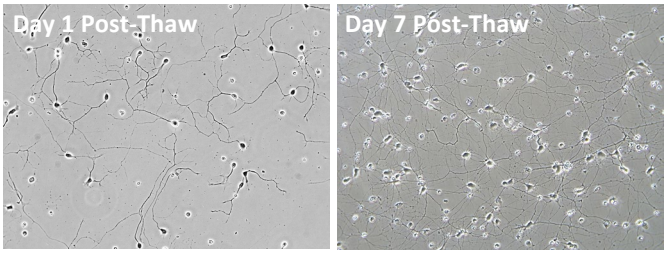
Maintenance of RealMOTO™

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.
4. After 7 days of maturation, RealSCP™ Schwann cell Precursors can be seeded

Figure 1: Typical Results from RealMOTO™



Preparing Moto-SCP Co-culture Kit (Co-culture Medium)

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

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

Formulating Co-Culture Medium with Rock Inhibitor for Thawing SCPs

1. Calculate the appropriate amount co-culture media necessary for thaw
2. Combine complete Moto-SCP Co-culture Medium with 10 uM Y-27632
3. Alternatively, combine complete SCP-MM medium with 50 nM Chroman-1

Thawing the RealSCP™ Schwann cell Precursors

1. Warm 6 mL DMEM/F12 to room temperature and add in 0.5% HSA to the mixture.

HSA in the wash medium is required to ensure the cells will pellet

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 a 37°C water bath until only remnant ice remains in the vial. Do not submerge the vial.
4. Ethanol-spray the vial and transfer the vial it into a laminar flow hood.
5. Once thawed completely, gently transfer the cells into a sterile 50 mL centrifuge tube.
6. Gently rinse the cryovial with 1 mL of warmed DMEM/F12 and transfer to the sterile centrifuge tube
7. Add 5 mL of warmed DMEM/F12 dropwise with agitation to the cell suspension. Transfer the culture to a 15 mL centrifuge tube to pellet.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Seeding RealSCP™

Anatomic recommends a general seeding density of 5K cells/cm2, but this is highly dependent on your assay of interest and time points to test. Please contact Technical Support for assay-specific seeding recommendations.

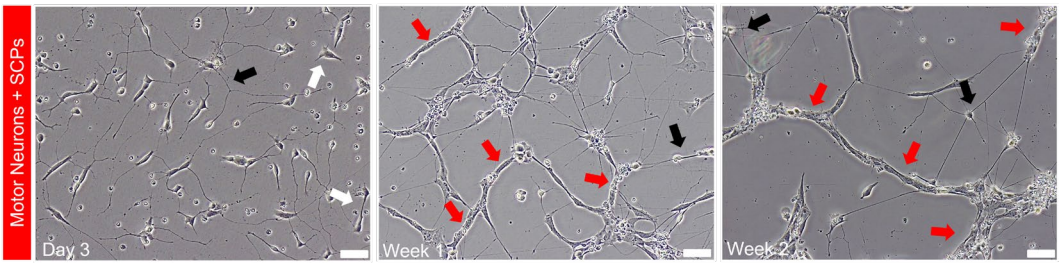
1. After the centrifugation, check the clarity of supernatant and visibility of a compact pellet. Aseptically withdraw the supernatant with a serological pipet without disturbing the cell pellet.
2. Cap tube and gently flick pellet so that it smears in the conical. Gently resuspend the cell pellet in 2 mL of Moto-SCP Co-culture Medium + Rock Inhibitor 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 2/3 of media from the culture vessel(s). Immediately add the appropriate volume of Moto-SCP Co-culture Medium + Rock Inhibitor.
5. Transfer the Schwann cell precursors 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 RealMOTO™ & RealSCP™ Co-Cultures

Avoid dislodging the RealSCP™ Schwann Cell Precursors by dispensing medium GENTLY as the cells can easily detach during culture handling.

1. Perform a **150%** media exchange with Moto-SCP Co-culture Medium the day after plating.
2. Perform a **2/3** media exchange with Moto-SCP Co-culture Medium two days after (Day 3).
3. Perform a **2/3** media exchange with Moto-SCP Co-culture Medium every 2 days (ie. Monday, Wednesday, and Friday). Cultures can be fed two-days in a row to align with this MWF schedule.
4. Culture the cells at 37°C, 5% CO2, and 95% humidity.

Figure 2: Typical Results from RealMOTO™ & RealSCP™ Co-Cultures



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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
Beta III Tubulin	1:250	Millipore	MAB1637
Choline Acetyltransferase	1:100	Millipore	AB144P
S100b	1:500	Sigma Millipore	S2532
SOX10	1:250	R&D Systems	AF2864
MPZ	1:250	Aves Labs	PZO-0020
MBP	1:100	Millipore	MAB386
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: Typical Results from RealMOTO

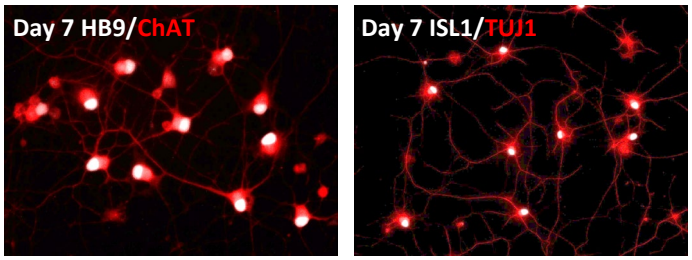
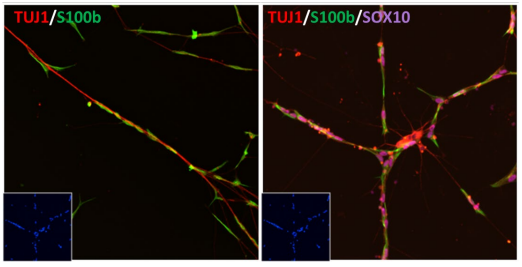


Figure: Typical Results from Co-Cultures



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