RealSCP Schwann Cell Precursors Quick Guide

ANATOMIC

Handling and Storage

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

Component (Items sold Separately)	Catalog #	Storage Temperature	Amount
RealSCP™ Schwann cell Precursors	4020	Liquid N_2	
RealSCP Maturation Medium	4030		
Basecamp Maturation Basal		4°C	115 mL bottle
RealSCP Maturation 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	-
Human Serum Albumin (HSA)	InVitria	777HSA017S
dPBS (-/-)	Various	-
DMEM/F12	Gibco™	11330057
Y-27632 (Dihydrochloride)	StemCell	72304
Chroman-1	Medchem Express	HY-15392

Preparing Cell Culture Surface

For most applications, use TC-treated cell culture vessels pre-coated with 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. Dilute iMatrix-511 SILK based on plate format into dPBS (-/-)
2. Add iMatrix-511 SILK to tissue culture-treated vessels.
3. Incubate the vessel overnight at 4°C or at least three 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 SCP-MM supplement at room temperature
2. Add this 5 mL supplement to the 115 mL Basecamp Basal bottle
3. **Store SCP-MM at 4°C for only up to 1 week.**
4. For long term storage, aliquot remaining Chrono™ SCP-MM into appropriate amounts to store at -20°C.
5. Equilibrate SCP-MM to room temperature before use.

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

Formulating SCP-MM with Rock Inhibitor for Thawing SCPs

1. Calculate necessary amount of SCP-MM needed for 1 day of culture
2. Combine complete SCP-MM with 10 uM Y-27632 for 1 day of culture
3. Alternatively, combine complete SCP-MM medium with 50 nM Chroman-1

Thawing the Cells

1. Warm 6 mL DMEM/F12 to room temperature and add in 0.5% HSA to the mixture.
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 + 0.5% HSA and transfer to the sterile centrifuge tube
7. Add 5 mL of warmed DMEM/F12 + HSA dropwise to the cell suspension in the centrifuge tube.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Plating out RealSCP™

Anatomic recommends a general seeding density of 5K-15K 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.

Culture Vessel	Seeding Volume	Seeding Density ($cells/cm^2$)	Cells/Well
6-well Plate	2 mL	5K-15K	48K-144K
12-well Plate	1 mL	5K-15K	19K-57K
24-well Plate	500 uL	5K-15K	9.5K-28.5K
96-well Plate	100 uL	5K-15K	1.65K-5K
384-well Plate	50 uL	10K	1K

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 SCP-MM + 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 iMatrix-511 SILK from the culture vessel(s). Immediately add the appropriate volume of SCP-MM + Rock Inhibitor. Do not let the coating dry out during the process.
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 Cells

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 Chrono™ SCP-MM the day after plating.
2. Perform a **2/3** media exchange with Chrono™ SCP-MM two days after (Day 3).
3. Perform a **2/3** media exchange with Chrono™ SCP-MM every 2 days (ie. Monday, Wednesday, and Friday).
4. Culture the cells at 37°C, 5% CO2, and 95% humidity.

Contacting Technical Support

Email: support@anatomic.com
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Version 1.2

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Expansion of RealSCP™

RealSCP™ can be expanded for up to a week in vitro before being cryopreserved or utilized in different assays. Anatomic recommends a general seeding density of 5-10K cells/cm^2 in a flask format. Over one week in vitro, the cells expand 2-3x the original seeding amount. Please contact Technical Support for assay-specific seeding recommendations.

1. Dilute iMatrix-511 into dPBS (-/-) and coat tissue culture-treated vessels for the proper amount of time as previously described.
2. Calculate the necessary amount of SCP-MM needed for 1 day of culture and combine with 10 uM Y-27632 OR 50 nM Chroman-1.
3. Thaw the cells as previously described.
4. Plate out RealSCP™ into the desired tissue culture flask as previously described.

Culture Vessel	iMatrix 511-SILK Dilution	Coating Volumes	Seeding Volume	Seeding Density (cells/cm^2)	Cells/ Well
T25 Flask	1:100	2.5 mL	5 mL	5K-10K	125-250K
T50 Flask	1:100	5 mL	10 mL	5K-10K	250K-500K
T75 Flask	1:100	7.5 mL	15 mL	5K-10K	375K-750K

Maintenance of Cells in a Tissue Culture Flask

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

1. Perform a **100%** media exchange with Chrono™ SCP-MM the day after plating.
2. Perform a **100%** media exchange with Chrono™ SCP-MM two days after (Day 3).
3. Perform a **100%** media exchange with Chrono™ SCP-MM every 2 days (ie. Monday, Wednesday, and Friday).
4. Culture the cells at 37°C, 5% CO2, and 95% humidity.

Phase Images of RealSCP Expansion

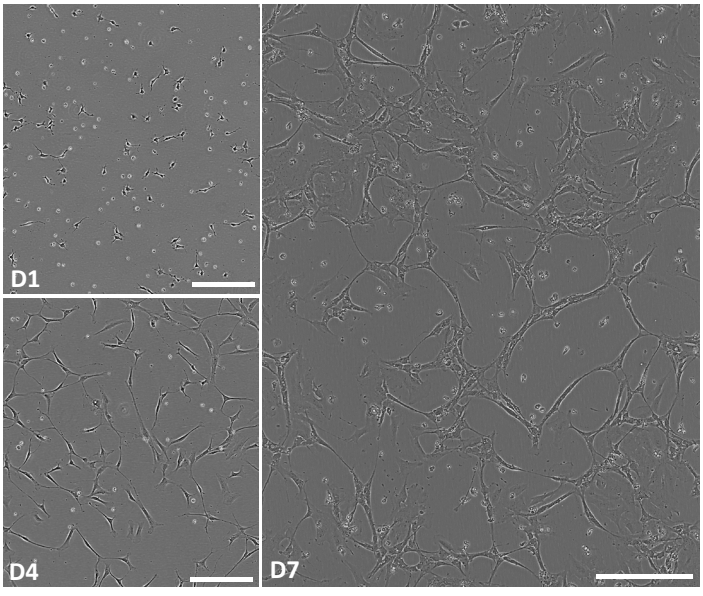

Figure. Phase images of RealSCP expansion in SCP-MM. Cells were plated at 10,000 cells/cm^2. Scale bars = 100 um.

Reagents Needed for Dissociation and Cryopreservation

Component	Vendor	Catalog #
Accumax	Innovative Cell Technologies	AM105
EDTA (0.5 mM)	Various	-
Human Serum Albumin (HSA)	InVitria	777HSA017S
dPBS (-/-)	Various	-
DMEM/F12	Gibco™	11330057
CryoStor® CS10	BioLife Solutions	210102
Cryo-vials of choice	Various	-
Cellular Freezing Container of choice	Various	-

Dissociation of RealSCP™

1. Prepare dissociation solution, composed of 10% Accumax diluted in 0.5 mM EDTA solution. The total volume needed for each tissue culture flask is the same as the coating volume for the corresponding culture volume.
2. Aspirate medium from the tissue culture vessel.
3. Wash 1x with half the prepared dissociation solution (i.e. the volume corresponding to the coating volume for each culture vessel).
4. Aspirate dissociation wash.
5. Add in the remaining half of the prepared dissociation solution and incubate the cells at 37 ° C for 10-20 minutes.

The cells should naturally release from the tissue culture flask. If there are some remaining cells attached, gently tapping on the bottom of the flask will help dislodge the cells.

6. After cells have dissociated from the tissue culture vessel, transfer the cell suspension into a 15 mL conical tube.
7. Fill the tube up to 15 mL with DMEM/F12 + HSA.
8. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

After this step, the RealSCP™ can either be cryopreserved and stored in liquid nitrogen for later use or replated at the desired seeding density with RealDRG™ for co-culture.

Cryopreservation of RealSCP™

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 the appropriate volume of CS-10 to create a smooth cell suspension at 1 million cells per mL.
3. Add 1 mL of cell suspension per cryo-vial.
4. Add vials to cellular freezing container and follow its' associated freezing protocol.
5. Store cells long term in liquid nitrogen.

Co-cultures of RealSCP™ with Motor Neurons

RealSCP and RealMOTO should be seeded at the same time from thaw.
For full details, reference 3032_Anatomic_MotoSCP 2D_QuickGuide.

Co-cultures of RealSCP™ with Sensory Neurons

Thaw and mature RealSCP for one week.
Thaw RealDRG and passage RealSCP together.
For full details, reference 1036_Anatomic_SensoSCP 2D_QuickGuide.

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