RealDRG™ Nociceptors Quick Guide

ANATOMIC

Handling and Storage

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

Component	Catalog #	Storage Temperature
RealDRG™ Nociceptors	1020	Liquid Nitrogen
Chrono™ Senso-MM	1030	-20°C
iMatrix-511 SILK	M511S	4°C

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 the appropriate amount of Chrono™ Senso-MM for the week at room temperature or overnight in the refrigerator
2. **Store Chrono™ Senso-MM at 4°C for only up to 1 week.**
3. For long term storage, aliquot remaining Chrono™ Senso-MM into appropriate amounts to store at -20°C.
4. Equilibrate Chrono™ Senso-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 RealDRG™

Anatomic recommends a general seeding density of 20K-25K cells/cm2, but this is highly dependent on your assay of interest and time points to test. 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-25K	192K-240K
12-well Plate	1 mL	20K-25K	70K-87.5K
24-well Plate	500 uL	20K-25K	38K-47.5K
96-well Plate	100 uL	20K-25K	6.4K-8K
384-well Plate	50 uL	60K	6K

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 Chrono™ Senso-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 Chrono™ Senso-MM. Do not let the coating dry out during the process.
5. Transfer the sensory 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 RealDRG™ Nociceptors by dispensing medium GENTLY as the cells can easily detach during culture handling.

1. Perform a **150%** media exchange with Chrono™ Senso-MM the day after plating.
2. Perform a **2/3** media exchange with Chrono™ Senso-MM every 2 days (ie. Monday, Wednesday, and Friday).
3. Culture the cells at 37°C, 5% CO2, and 95% humidity.

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