Quick Guide
RealDRG™ Nociceptors

Handling and Storage

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

Component	Storage Temperature
RealDRG™ Nociceptors	Vapor Phase of Liquid Nitrogen

Other Reagents Needed

Component	Vendor	Catalog #
Chrono™ Senso-MM	Anatomic	7008
iMatrix-511 SILK	Anatomic	M511S
96-Well Polystyrene Plates	FischerScientific	12-565-501
Poly-L-Ornithine Solution (0.01%)	Sigma-Aldrich	A-004-C
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. Please contact Technical Support for assay-specific cell culture surface recommendations.

1. Thaw Poly-L-Ornithine solution at room temperature.
2. Fully coat the cell culture surface with diluted Poly-L-Ornithine solution. Use 70 uL volume for 96 well plates.
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 with sterile water.
5. Dilute iMatrix-511 SILK 1:50 into dPBS (-/-)
6. Add iMatrix-511 SILK to tissue culture-treated vessels. Use 70 uL volume for 96 well plates.
7. 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 the appropriate amount of Chrono™ Senso-MM for the day at room temperature or overnight in the refrigerator
2. Store Chrono™ Senso-MM at 4°C for 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.

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 the Cells

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. Anatomic recommends a seeding density of 10-20K cells/cm2 for fluorescence based or 30K-60K cells per well for 96 well plates.
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.

Culture Vessel	Surface Area	Plating Volume	Cell Number
6-well Plate	9.6 cm2	2 mL	100K-200K
96-well Plate	0.32 cm	100 uL	30K-60K

Maintenance of Cells

Avoid dislodging the RealDRG™ Nociceptors by dispensing medium gently as the cells can easily detach during culture handling

1. Add 50% plating volume more Chrono™ Senso-MM the day after plating. For 96 well plates, add 50 uL of media.
2. Replace 2/3 of the Complete Maintenance Medium every 2-3 days.
3. Culture the cells at 37°C, 5% CO2, and 95% humidity.

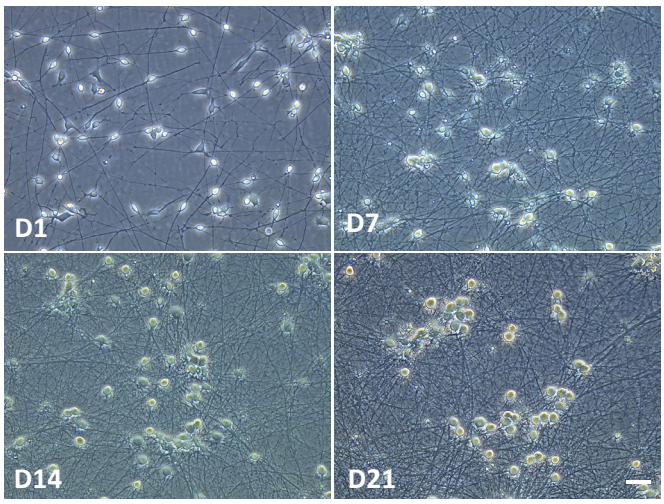

Figure 1. RealDRG™ Nociceptor time course morphology. Scale bar 50 um

Contacting Technical Support

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ANATOMIC

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Immuncytochemistry

Reagents Needed

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
Primary antibodies (ie TUJ1)		MAB1637
Secondary antibodies		
Water for Cell Culture	Life Technologies	A1287303
DAPI Dilactate	Life Technologies	D3571

Permeabilization Buffer:
0.27% (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 ensure 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 96-well is 150 uL. Do not remove media.
2. Add 150 uL Formalin to culture medium
3. Incubate room temperature 10 minutes
4. Aspirate 250 uL Formalin

Permeabilization:
1. Add 150 uL Permeabilization Buffer
2. Incubate room temperature 10 minutes
3. Aspirate 150 uL Permeabilization Buffer

Intracellular Primary, Secondary, Counterstain
1. Add 50 uL diluted primary antibodies in blocking buffer
2. Parafilm-wrap edges of tissue culture vessel
3. Incubate overnight 4C
4. Aspirate 50 uL diluted primary antibodies
5. Add 150 uL Blocking Buffer
6. Aspirate 150 uL Blocking Buffer
7. Add 50 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 50 uL diluted secondary antibodies and DAPI
11. Add 250 uL dPBS
12. Aspirate 150 uL dPBS
13. Add 150 uL dPBS
14. Aspirate 150 uL dPBS
15. Add 150 uL dPBS
16. Aspirate 150 uL dPBS
17. Parafilm-wrap edges of tissue culture vessel
18. Store 4C in darkness for downstream imaging

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