FLIPR 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
Chrono™ Senso-MM	-20°C
iMatrix™ 511-SILK	4°C

Other Reagents Needed

Component	Vendor	Catalog #
384-well plate	Greiner	781091
Poly-L-Ornithine Solution (0.01%)	Sigma-Aldrich	A-004-C
dPBS (-/-)	Various	-
DMEM/F12	Gibco™	11330057
Calcium 6 Assay Kit	Molecular Devices	R8194

(!) ***To decrease well-to-well variability and prevent evaporation, do not use the outer two rows and columns of the 384-well plate. Leave these wells for a 80 uL water barrier.***

Preparing Cell Culture Surface

1. Thaw Poly-L-Ornithine solution at room temperature.
2. Fully coat the cell culture surface with Poly-L-Ornithine solution. Use 20 uL volume for 384 well plates.
3. Add 100 uL of water to the two rows and columns surrounding the wells used.
4. Parafilm and allow cell culture vessel to sit at room temperature overnight.
5. Aspirate the Poly-L-Ornithine solution the following day and rinse 384-well plate with 70 uL sterile water.
6. Dilute iMatrix™ 511-SILK 1:25 into dPBS (-/-)
7. Add iMatrix™ 511-SILK to 384 well plate. Use 20 uL volume for 96 well plates.
8. Incubate the plate for 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 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. Sample to perform a viable cell count
9. Centrifuge the cell suspension at approximately 300 × g for 4 minutes.

Plating the Cells

1. Anatomic recommends a seeding density of 6K cells per 384-well (~108K cells/cm2) into 50 uL of Chrono™ Senso-MM.
2. After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically aspirate the supernatant without disturbing the cell pellet.
3. Cap tube and gently flick pellet so that it smears in the conical. Gently resuspend the cell pellet into Chrono™ Senso-MM complete growth medium to create a smooth cell suspension.
4. Remove iMatrix™ 511-SILK from the culture vessel(s). Immediately add 50 uL of cell suspension. Do not let the coating dry out during the process.
5. Tap the culture vessel(s) back and forth to ensure even plating of cells.
6. Place cultures onto a flat surface for 10 minutes to allow cells to settle evenly
7. Transfer plate into the incubator at 37°C, 5% CO_2, and 95% humidity.

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 384-well plates, add 25 uL of media.
2. Gently replace 2/3 of the Complete Maintenance Medium (50 uL) every 2-3 days. An ideal feeding schedule would be Monday, Wednesday, and Friday.
3. Culture the cells at 37°C, 5% CO_2, and 95% humidity.

(!) ***At the end of each week, combat evaporation by refilling the water barrier to the appropriate levels. If media levels drop, add additional media to appropriate levels.***

Preparation of Loading Buffer:

This protocol is an adapted version of Molecular Device's Calcium 6 product manual which can be found here.

1. Remove one vial of Component A from the freezer and equilibrate to room temperature.
2. Equilibrate Component B to room temperature (You can substitute 1X HBSS Buffer plus 20 mM HEPES for Component B.)
3. Dissolve the contents of one Component A vial by adding 10 mL of Component B and then mix by vortexing for ~1 to 2 minutes until the contents of the vial are dissolved.

Loading the Dye:

1. Gently remove existing medium from the well.
2. Immediately add 20 uL of Component B (assumed to be 1X HBSS Buffer plus 20 mM HEPES) to each well

(!) ***Go row by row for the above steps to ensure cells minimize time without media or buffer to prevent drying out.***

3. Add 20 uL of prepared Loading Buffer.
4. Incubate for 2 hours at 37 °C.
5. Cells are now ready for imaging

Contacting Technical Support

Email: info@anatomic.tech
Phone: 612-208-6735