# Calcium Imaging Quick Guide
## RealDRG™ Nociceptors

**Reagents Needed**

| Component | Vendor | Catalog # |
|---|---|---|
| Calbryte™ 520 AM | AAT Bioquest | 20651 |
| PowerLoad™ Concentrate, 100X | Thermo Fisher | P10020 |
| DMEM/F12 (Imaging Medium) | Gibco™ | 11330057 |

***This protocol is an adapted version of AAT Bio's Calbryte™ 520 AM product manual which can be found here.***

**Resuspending Calbryte 520 AM:**

1. Briefly centrifuge 50 µg tube
2. Resuspend in 18 uL DMSO to make a ~2.5 mM (500X) stock solution
3. Store leftover solution at -20°C

**Preparing 2X Loading Solution:**

1. Calculate the appropriate amount of 2X Loading Solution needed
2. Add 1:250 Calbryte™ 520 AM
3. Add 1:50 PowerLoad™ Concentrate

***Notes: 1) The resulting concentration of Pluronic F-127 in PowerLoad™ Concentrate is unknown. 2) We do not use Probenecid due to potentially toxic effects on the cell and the Calbryte protocol states that the calcium indicator was specifically designed to eliminate the need for probenecid.***

4. Vortex
5. Add appropriate amount of DMEM/F12

**Loading the Dye:**

1. Remove half the existing medium from the well. For 96-well plates, leave 50 uL of medium remaining.
2. Add the same amount removed of 2X Loading Solution. For 96-well plates, add 50 uL of Loading Solution.

***Note: The final concentration of Calbryte™ 520 AM should be 5 uM and PowerLoad™ Concentrate 1X.***

3. Incubate for 40-50 minutes at 37 °C.

***Note: Incubation for 30 and 70 minutes also works, but signal and background may de/increase.***

4. Exchange the Loading Solution with DMEM/F12 (imaging medium) twice. For 96-well plates, add 90 uL of DMEM/F12.
5. Incubate the cells for 15 minutes at room temperature.

***Note: This "incubation" time typically occurs while setting up everything at the microscope, finding a sweet spot of cells, adjusting focus, etc.***

**Imaging Setup**

Fluorescent videos of RealDRG™ cultures were acquired using a Leica DMI6000B microscope, a DFC365FX camera, and a 10x air objective (NA 0.25, Leica Microsystems) for a duration of 185 seconds at 4 frames per second.

**Drug Dosing:**

1. Drugs were prepared 10X working concentration in 1% DMSO in DMEM/F12.
2. Image acquisition was started on a random field of view with >200 cells.
3. Within 10 seconds of starting image acquisition, drugs were added to the cultures. For 96-well plates, 10 uL of the drug was added.

**Compound List:**

| Drug | Working Conc. | Vendor | Catalog # |
|---|---|---|---|
| Veratridine | 1 µM | Tocris | 2918 |
| Tetrodotoxin | 1 µM | Biotium | 00061 |
| KCl | 30 mM | Millipore Sigma | P5405 |
| Capsaicin* | 100/500 nM | Sigma-Aldrich | M2028 |
| Capsazepine* | 1 µM | Tocris | 0464 |
| Menthol* | 100 µM | Sigma-Aldrich | M2772 |

***Note: RealDRG™ Nociceptors only respond to the compounds marked with an asterisk after a minimum of 4 weeks of maturation in Senso-MM.***

**Image Analysis:**

Analysis was automated using FIJI

1. Individual somata were detected by local maxima-based image segmentation and binary threshold
2. Mean intensity was measured over time inside each soma
3. The intensity profiles were graphed, in terms of ΔF/F0, where ΔF is the intensity differential compared to minimum fluorescence (F0) of the given sample