FLIPR FMP Blue Assay

1. Make2 L Locke's buffer (with glycine for neurons). pH to 7.4.

Locke's buffer

Reagent	mM	1 Liter	2 Liters
HEPES	8.6	2.144 g	4.288 g
KCl	5.6	0.417 g	0.834 g
NaCl	154	9.0 g	18.0 g
Glucose	5.6	1.009 g	2.018 g
$MgCl_2$	1.0	0.204 g	0.408 g
$CaCl_2$	2.3	0.338 g	0.676 g
Glycine (primary neurons)	0.1	7.5 mg	15 mg
Probenecid (CHO Ca^{2+} assays)		0.1175 g	0.235 g

2. Follow the steps on the machine for turning on and calibrating the machine.
3. To calibrate the machine, perform a yellow plate reading. First run the test without selecting calibration. Percent standard deviation will be high. Next run the calibration step. Percent standard deviation should be ~1.2%. Document max, average, min value and % standard deviation.
    a. Filter1 for yellow plate reading and calcium oscillations
    b. Filter2 for FMP blue.
4. Plan your experiment using 96-well plate template.
5. Create a file for each day experiments are performed. It is saved in Documents→Pierce→ DATE
6. Select your experiment. If template is not made, will have to generate template.
7. To make FMP blue, add 1 FLIPR membrane potential assay kit to 20 mL Locke's buffer. Incubate 100 uL on cells for 45 minutes prior to experimentation.
8. Dilute out the drug(s).
    a. For FMP blue, will do 100:20 uL
        i. -5 dilution is 1.2 uL drug (50 mM stock) in 1 mL FMP dye.
        ii. Serial dilute in 100 uL drug into 900 mL FMP dye.
9. Run on FLIPR2.
10. Analyze using GraphPad prism

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