| Khurana Lab<br>BRIGHAM AND WOMEN'S HOSPITAL<br>HARVARD MEDICAL SCHOOL | SOP-VK_00xxxx | **Fluo-4 Calcium Imaging** | Author:<br>Edinson Lucumi Moreno<br>Approved: | Revision | Issued<br>2019-10-25<br>Revised |
|---|---|---|---|---|---|

1. **Purpose**
   To measure the firing activity of cultured iPSC derived neurons
2. **Scope**
   This procedure is used to prepare cultured neurons for Calcium imaging
3. **Materials**
   96 well plate with differentiated neurons
4. **Reagents**
   Neurobasal medium no phenol red (cat #: 12348017)
   Fluo-4-AM (cat #: F14201)
   DPBS

| Reagent | Reference | Mol weight (mg/mmol) | Solvent | [Stock mM] | [Working conc. µM] | Medium |
|---|---|---|---|---|---|---|
| Fluo-4-AM | F14201 | 1096.95 | DMSO | 1 | 5 | Neurobasal |

5. **Procedure for Calcium Fluo-4**
   5.1 Prepare 1mM Fluo-4 stock solution by adding 45µL of DMSO to vial
   5.2 Prepare 5µL aliquots of 1mM Fluo-4 stock in PCR Eppendorf tubes and store them at -20 °C
   5.3 Take one 5µL aliquot of 1mM Fluo-4 and transfer to 995µL of neurobasal medium (work at low light)
   5.4 Transfer medium from selected wells of the 96 well plate with differentiated neurons. to an Eppendorf tube
   5.5 Wash well by adding 150µl of neurobasal medium, be careful not to detach of perturb the neurons
   5.6 Repeat the washing step 1 more time
   5.7 Add 120µl of Fluo-4 AM [5µM in neurobasal medium] into wells
   5.8 Incubate for 20 min
   5.9 Remove the neurobasal medium with Fluo-4 AM, by transferring it to a waste container
   5.10 Wash well by adding 150µl of neurobasal medium, be careful not to detach of perturb the neuron
   5.11 Remove the neurobasal medium to waste
   5.12 Add the old differentiation medium to the same well you took them from.
   5.13 Incubate for 10 min
   5.14 Acquire time lapse images in Nikon microscope using the Fluo-4 Calcium imaging acquisition protocol