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# Fluo-4 Calcium Imaging

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1 Works for me

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[dx.doi.org/10.17504/protocols.io.bu37nyrn](https://dx.doi.org/10.17504/protocols.io.bu37nyrn)

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## ABSTRACT

This protocol details the measurement of the firing activity of cultured iPSC derived neurons. This procedure is used to prepare cultured neurons for Calcium imaging.

## ATTACHMENTS

[dh4bbiq7.pdf](#)

## DOI

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## PROTOCOL CITATION

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## KEYWORDS

Fluo-4 Calcium Imaging, iPSC derived neurons

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Aug 19, 2021

## OWNERSHIP HISTORY

May 17, 2021 Urmilas

Jun 23, 2021 hendersa

## PROTOCOL INTEGER ID

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## MATERIALS TEXT

### Materials:

96 well plate with differentiated neurons.

### Reagents:

 Neurobasal™ Medium, minus phenol red **Thermo**

**Fisher Catalog #12348017**

 Fluo-4, AM, cell permeant **Thermo**

**Fisher Catalog #F14201**

DPBS

A	B	C	D	E	F	G
Reagent	Reference	Mol weight (mg/mmol)	Solvent	[Stock mM]	[Working conc. $\mu$ M]	Medium
Fluo-4-AM	F14201	1096.95	DMSO	1	5	Neurobasal

### Procedure for Calcium Fluo-4

30m

1 

Prepare **1 Milimolar (mM)** Fluo-4 stock solution by adding **45  $\mu$ l** of DMSO to vial.

2 Prepare **5  $\mu$ l** aliquots of **1 Milimolar (mM)** Fluo-4 stock in PCR Eppendorf tubes and store them at **-20 °C**.

3 Take one **5  $\mu$ l** aliquot of **1 Milimolar (mM)** Fluo-4 and transfer to **995  $\mu$ l** of neurobasal medium (work at low light).

4 Transfer medium from selected wells of the 96 well plate with differentiated neurons to an Eppendorf tube.

5 

Wash well by adding **150  $\mu$ l** of neurobasal medium, be careful not to detach or perturb the neurons.

6 

Repeat the washing step 1 more time.

7 

Add  **120  $\mu$ l** of Fluo-4 AM [**5 Micromolar ( $\mu$ M)** in neurobasal medium] into wells.

8 

20m

Incubate for  **00:20:00** .

9 Remove the neurobasal medium with Fluo-4 AM, by transferring it to a waste container.

10 

Wash well by adding  **150  $\mu$ l** of neurobasal medium, be careful not to detach or perturb the neuron.

11 Remove the neurobasal medium to waste.

12 Add the old differentiation medium to the same well you took them from.

13 

10m

Incubate for  **00:10:00** .

14 

Acquire time lapse images in Nikon microscope using the Fluo-4 Calcium imaging acquisition protocol.