Technical Manual

GenieHTS Calcium Flux Ratiometric Assay Kit

- **Catalogue Code: ASIB010**
- **Size: 10 plates**
- **Research Use Only**

Introduction

The Assay Genie Calcium Flux Ratiometric kit provides the necessary reagents for conducting no wash, ratiometric calcium flux assays compatible with plate reader and fluorescence microscopy applications. Individual components are provided to give the user the flexibility needed to customize your assay.

Fura-2 is the most popular UV-excitable, ratiometric green indicator for intracellular calcium (Ca^{2+}) measurements. Ex/Em: 340/505 nm can be used to measure Ca^{2+} -bound Fura-2, and Ex/Em: 380/505 nm can be used to detect Ca^{2+}-free Fura-2. Ratiometry is not only optimal for imaging applications where quantification of intracellular Ca^{2+} concentrations is desired, it also reduces effects of photobleaching, heterogenous dye loading, and variable cell morphology.

When following the recommended protocol, GenieHTS Calcium Flux Ratiometric provides enough reagents to make 100 mL of working solution, enough for ten 96-well plates. The actual number of assays will vary according to optimal dye concentrations for your application.

Kit Features:

- **Excitation:** 340 (UV)
  380nm
- **Emission:** 505 nm
- **Kd:** 145 nM
- **MW:** 1002
- **Solubility:** DMSO
- **Purity:** >95%

Kit Components

Component Name	Size	Storage
Fura-2 AM (50 µg vial)	Lyophilized (10)	-20°C
50X TRS solution	2 mL	4°C
100X Pluronic F-127 solution	1 mL	4°C
1X Assay Buffer	100 mL	4°C

Materials needed but not provided

- Compounds to be tested.
- Buffers and solvents for dissolution.
- Reagents necessary for cell culture.
- A fluorescence or plate reader ~ 340 nm /~ 380 nm and 505 nm.

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Assay Notes:

1. Optimal dye concentrations will vary depending on cell type and application. Recommended dye concentrations range between 1 µM and 10 µM.
2. Aqueous solutions of Fura-2 AM are susceptible to hydrolysis; therefore, all working solutions should be used as quickly as possible and no later than 2 hours after preparation for best results. Alternatively, prepared dye loading solution can be frozen and stored for up to 1 week.

Assay Procedure:

The following protocol provides general guidelines for using this dye to measure intracellular calcium. All loading conditions (dye concentration, temperature, and time) should be optimized for your specific assay, application, and instrumentation.

1. Allow all reagents to warm to room temperature before proceeding.
2. Add 9.7 mL of 1X Assay Buffer to a conical tube (15-50 mL).
3. Add 100 µL of 100X Pluronic F-127 solution to conical tube. Pluronic F-127 is a biocompatible surfactant that aids in dye dissolution, ensuring equitable dye distribution and cellular loading.
4. Add 200 µL of 50X TRS solution to conical tube. TRS is a membrane impermeant dye useful for masking extracellular fluorescence.[1]
5. Vortex conical tube briefly to mix.
6. Dissolve Fura-2 AM in 25 µL of DMSO. After adding DMSO, vortex tube briefly to dissolve the indicator dye, then centrifuge briefly to collect all contents at the tube bottom. Add entire contents of indicator dye tube to assay buffer solution to make a dye loading solution.
7. Vortex dye loading solution briefly to mix.
8. Remove the cell culture medium from your cells and add dye loading solution. Recommended volumes are: 35 mm dish or 6-well plate, 1.5 mL; 96-well plate, 100 µL; 384-well plate, 20 µL.[2]
9. Incubate in a cell culture incubator at 37°C for 60 minutes.
10. Acquire data using a kinetic plate reader (Ex/Em: 340 and 380 nm/505 nm) or image using a fluorescence microscope (using filters for Fura).[3] Begin data acquisition at a 1 Hz frequency, then after 10 seconds add your compounds of interest to the cell-containing plate and continue data acquisition for an additional 90 seconds.

1. Caution is advised when using TRS as it may have undesirable effects on assay performance for the target of interest.
2. In some cases, such as when using suspension-based cultures, medium aspiration is not desirable. In these circumstances, we recommend doubling the concentration of all reagents in your dye loading buffer and adding an equal volume of dye loading solution to medium to achieve the same final loading concentrations.
3. Fura-2 fluorescence is temperature sensitive. Therefore, it is important to maintain a stable temperature during data acquisition. If you want to conduct assays at room temperature, allow your plate to cool on the bench for 20 minutes prior to reading plate.

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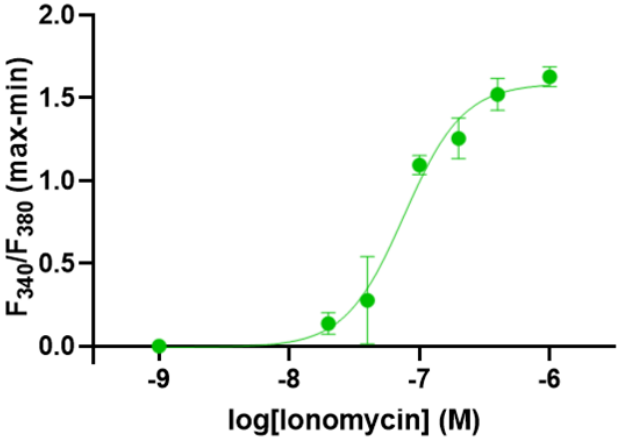

Figure 1. Ionomycin was used to generate a concentration response curve in HeLa cells using Calcium Flux Ratiometric kit. Data was acquired using a Cytation 5 and Prism software was used to calculate the ratio of F_{340}/F_{380}. All measurements were recorded at 37°C.

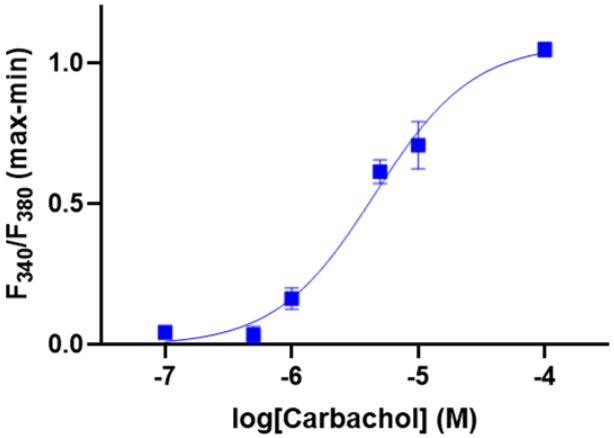

Figure 2. Carbachol was used to stimulate endogenous muscarinic M3 receptor in HEK 293 cells. Data was acquired using a Cytation 5 and Prism software was used to calculate the ratio of F_{340}/F_{380} before and after the addition of carbachol. The EC_{50} is 4.5 µM. All measurements were recorded at 37°C.

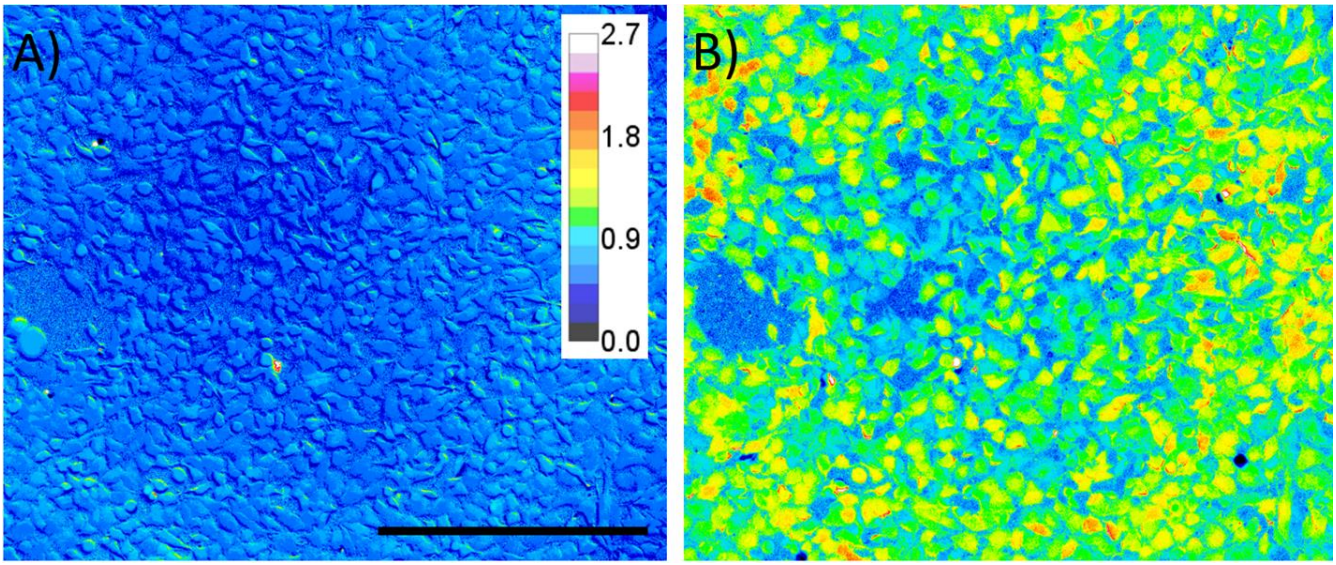

Figure 3. Ratiometric imaging of calcium flux using Calcium Flux Ratiometric Assay. Images of the same region of interest (ROI) were acquired using a 10X objective, excitation filters: (334/40 nm) and (387/11 nm), dichroic mirror (FF409-DiO3), and a single emission filter - (510/80 nm). Corresponding fluorescence images were divided by each other in ImageJ to produce a single image where the colorbar intensity represents the ratio of F340/F380. A) Image of HeLa cells prior to addition of ionomycin. B) Image of the same ROI of HeLa cells after the addition of 500 nM ionomycin. Scale bar - 500 µm.

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References

1. Neher E. *The use of fura-2 for estimating Ca buffers and Ca fluxes.* Neuropharmacology. (1995). 34(11).

2. Grynkiewicz G, et. al. *A new generation of Ca2+ indicators with greatly improved fluorescence properties.* J. Biol. Chem. (1985). 260(6).

3. Clementi EA, et al. *Monitoring changes in membrane polarity, membrane integrity, and intracellular ion concentrations in Streptococcus pneumoniae using fluorescent dyes.* JOVE. (2014). 84(e51008).

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