Technical Manual

GenieHTS Calcium Flux Assay Kit

- **Catalogue Code: ASIB001**
- **Size: 10 plates**
- **Research Use Only**

Introduction

The Assay Genie w is a total assay solution for multi-well plate-based, high-throughput measurements of changes intracellular Ca^{2+} mediated through a wide-variety of plasma membrane and intracellular calcium channels and transporters. The Assay Genie GenieHTS Calcium Flux Assay is also useful for investigating numerous effectors of ion channels and transporters including G protein-coupled receptors, lipid kinases and protein kinases. In multi-well, plate-based formats, the GenieHTS Calcium Flux Assay can be used to discover and characterize the effects of many tens-of-thousands of compounds and environmental factors on effectors of intracellular Ca^{2+}. For the last 25 years, fluorescence-based measures of Ca^{2+} flux have brought about the discovery of small-molecule modulators of a host of Ion channels, transporters, GPCRS and other targets of interest for both drug discovery and basic research. The Assay Genie GenieHTS Calcium Flux Assay provides all the reagents necessary for use as a washed or no-wash assay with adherent or non-adherent cells. The optional use of a probenecid solution and an extracellular background masking solution offers the ultimate in compatibility for cells types which are difficult to load with fluorescent Ca^{2+} indicators (e.g. Chinese Hamster Ovary, CHO cells) and when performing assays in complete, serum-containing cell culture medium is desired.

Kit Features:

- **Excitation:** 490nm
- **Emission:** 515nm
- **MW:** 1097

Kit Components

Table 1

Component Name	Size	Storage
GenieHTS Calcium Reagent	Lyophilized (10)	-20°C
DMSO	225µL	4°C
Dye Solvent	4mL	4°C
10X Assay Buffer	20mL	4°C
TRS	4mL	4°C
Probenecid Solution	4mL	4°C

Materials needed but not provided

- Compounds to be tested.
- Buffers and solvents for dissolution.
- Reagents necessary for cell culture.
- A fluorescence plate reader ~ 490 nm /~ 520 nM.
- Plate reader capable of collect kinetic data (1 Hz) e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR and Molecular Devices FlexStation.

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Assay Procedure

Adherent Cells: Wash Method

The instructions given below are for one, 384-well microplate. Certain aspects of the instructions may need to be altered, as appropriate, for multiple microplates or 2other assay formats (e.g. 96-well microplates or non-adherent cells). The Assay Genie GenieHTS Calcium Flux indicator and Calcium indicator-containing solutions should be protected from direct light.

1. Add 20 µL DMSO to the tube containing GenieHTS Calcium Reagent.
2. Vortex until the GenieHTS Calcium Reagent is fully dissolved.
3. Add water according to the below table to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Assay Buffer to tube from step 3.
5. Add 200 µL of Dye Solvent to the tube from step 4.
6. If desired add 200 µL of Probenecid Solution to the tube from step 5.

Table 2: Dye Loading Solution

Component	Method 1	Method 2
GenieHTS Calcium Indicator Solution	20µL	20µL
Dye Solvent	200µL	200µL
10X Assay Buffer	1mL	1mL
Probenecid Solution*	-	200µL
Water	8.8mL	8.6mL
Total	**10mL**	**10mL**

* Probenecid may be included in the Dye Loading Solution to aid dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). However, caution is advised when using Probenecid as it may have undesirable effects on assay performance for the target of interest.

7. Add 20 µL of GenieHTS Calcium Indicator Solution from step 2 to the tube from step 6.
8. Briefly vortex the tube from step 7 to mix.
9. Remove the cell-culture medium from the 384-well microplate containing the cells of interest.
10. Add 20 µL per well of the Dye Loading Solution from step 8 to the microplate from step 9.
11. Incubate the microplate containing the cells and Dye Loading Solution for 1 hour at 37°C.

Table 3: Wash Solution

Component	Method 1	Method 2	Method 3	Method 4
10X Assay Buffer	1mL	1mL	1mL	1mL
TRS*	-	200µL		200µL
Probenecid Solution	-	-	200µL	200µL
Water	9mL	8.8mL	8.8mL	8.6mL
Total	**10mL**	**10mL**	**10mL**	**10mL**

*TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.

12. Prepare Wash Solution in a 15 mL centrifuge tube by adding the appropriate amounts of water, 10X Assay Buffer and other components if desired as shown in Table 3.

13. Briefly vortex the tube from step 12 to mix.
14. Remove Dye Loading Solution from microplate in step 11.
15. Add 20 µL per well of the Wash Solution prepared in step 13 to the microplate from step 14.
16. Transfer the washed, dye-loaded, cell-containing microplate from step 15, along with an additional microplate containing a stimulus solution of interest, to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation).
17. Acquire data using an excitation wavelength of ~ 490 nm, an emission wavelength of ~ 520 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds add 5 µL of the stimulus solution to the cell-containing plate and continue data acquisition for an additional 90 seconds.**

    **The timing of and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.

Adherent Cells: No-wash Method

1. Add 20 µL DMSO to the tube containing GenieHTS Calcium Indicator.
2. Vortex until the GenieHTS Calcium Indicator is fully dissolved.

Table 4: Dye Loading Solution

Component	Method 1	Method 2
GenieHTS Calcium Indicator Solution	20µL	20µL
Dye Solvent	400µL	400µL
10X Assay Buffer	1mL	1mL
TRS*	400µL	400µL
Probenecid Solution**	-	400µL
Water	8.2mL	7.8mL
Total	**10mL**	**10mL**

*TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence. Caution is advised when using TRS or other extracellular masking solutions as they may have undesirable effects on assay performance for the target of interest.
**Probenecid may be included in the Dye Loading Solution to aid dye retention. This may be particularly important in certain cell lines (e.g. CHO cells). However, caution is advised when using Probenecid as it may have undesirable effects on assay.

3. Add appropriate volume of water (Table 4) to a 15 mL centrifuge tube.
4. Add 1 mL of 10X Assay Buffer to tube from step 3.
5. Add 400 µL of Dye Solvent to the tube from step 4.
6. Add 400 µL of TRS to the tube from step 5.
7. If desired add 400 µL of Probenecid Solution to the tube from step 6.
8. Add 20 µL of GenieHTS Calcium Indicator Solution from step 2 to the tube from step 7.
9. Briefly vortex the tube from step 8 to mix.
10. Add 20 µL per well of the Dye Loading Solution from step 9 to the cell-containing microplate. Do not remove the cell culture medium.
11. Incubate the microplate containing the cells and Dye Loading Solution for 1 hour at 37°C in a cell culture incubator.

12. Transfer the dye-loaded, cell-containing microplate from step 11, along with an additional microplate containing a stimulus solution of interest, to a kinetic-imaging plate reader (e.g. WaveFront Panoptic, Hamamatsu FDSS, Molecular Devices FLIPR or Molecular Devices FlexStation).
13. Acquire data using an excitation wavelength of ~ 490 nm, an emission wavelength of ~ 520 nm and an acquisition frequency of 1 Hz. Begin data acquisition and after 10 seconds add 10 µL of the stimulus solution to the cell-containing plate and continue data acquisition for an additional 90 seconds.**

**The timing of and volume of stimulus solution addition may vary. Some experiments may include the addition of other solutions to the cell-containing microplate prior to the addition of the stimulus solution. In these cases, the volume of the stimulus solution addition should be altered to account for the additional volume of solution in the cell-containing microplate.