

# Cell Meter™ Fluorescence Gap Junction Tracing Kit

 Catalog number: 23600  
 Unit size: 100 Tests

Component	Storage	Amount
Component A: Calcein UltraGreen™ AM	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: DiD	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: GAP Junction Assay Buffer	Freeze (< -15 °C)	1 bottle (100 mL)
Component D: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

## OVERVIEW

Gap junctions are specialized intercellular membrane channels constituted with connexin that selectively facilitate the passage of small molecules of <1.5 kD across cells. They are tightly regulated by voltage, growth factors, cAMP, and retinoids, and they are modulated by phosphorylation. Cell Meter™ Fluorescence Gap Junction Tracing Kit provides a reliable and robust assay for the in vitro determination of gap junction function. The method is noninvasive. The cell population under study is divided such that one fraction is loaded with a lipophilic cell plasma membrane permeable dye, Calcein UltraGreen™ AM, that is hydrolyzed upon cellular uptake by cytoplasmic esterases to yield Calcein UltraGreen, a highly fluorescent and well-retained and membrane-impermeable molecule. The other fraction is loaded DiD, which is a lipophilic membrane dye that diffuses laterally to stain the entire cell membrane in deep red fluorescence upon incorporation into membranes. The two fractions are mixed and incubated under coculture conditions. Calcein UltraGreen is transferred to the DiD-stained cells through gap junctions. The assessment of this uptake can be monitored by fluorescence imaging or flow cytometry.

## AT A GLANCE

### Protocol summary

1. Add Calcein Ultragreen AM working solution to the cells
2. Add DiD working solution to the cells
3. Incubate at 37 °C for 10-30 minutes
4. Wash cells with GAP Junction Assay Buffer
5. Resuspend cells in cell culture medium and mix them with 1:1 ratio
6. Measure the fluorescence signal at various times using a flow cytometer with 530/30 nm and 660/20 nm emission filters or a fluorescence microscope with FITC and Cy5 filter sets

### Important

Thaw all the kit components at room temperature before starting the experiment.

## KEY PARAMETERS

### Flow cytometer

Excitation	488 nm and 640 nm laser
Emission	530/30 nm and 660/20 nm filters
Instrument specification(s)	FITC and APC channel

### Fluorescence microscope

Excitation	FITC and Cy5 filter sets
Emission	FITC and Cy5 filter sets
Recommended plate	Black wall/clear bottom

## PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

### 1. Calcein Ultragreen AM stock solution

Add 50 µL of DMSO (Component D) into one Calcein Ultragreen AM vial (Component A) and mix well.

**Note** Store the unused Calcein Ultragreen AM stock solution at -20 °C in single use aliquots to avoid freeze thaw cycles.

**Note** One vial is enough for 50 tests.

### 2. DiD stock solution

Add 100 µL of DMSO (Component D) into DiD (Component B) and mix well.

**Note** Store the unused DiD stock solution at -20 °C in single use aliquots to avoid freeze thaw cycles.

## PREPARATION OF WORKING SOLUTION

### 1. Calcein Ultragreen AM working solution

Add 1 µL of Calcein Ultragreen AM stock solution to 1 mL of GAP Junction Assay Buffer and mix well.

**Note** Calcein Ultragreen AM working solution should not be stored and should be used promptly.

**Note** 1 mL Calcein Ultragreen AM working solution is enough for two tests.

### 2. DiD working solution

Add 1 µL of DiD stock solution into 1 mL of GAP Junction Assay Buffer and mix well.

**Note** DiD working solution should not be stored and should be used promptly.

**Note** 1 mL DiD working solution is enough for two tests.

## SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

### Cell staining protocol for Calcein Ultragreen AM

1. Grow cells in cell culture medium in 6-well cell culture plates.
2. Remove the cell culture medium and add 0.5 mL of Calcein Ultragreen AM working solution.
3. Incubate cells at 37 °C for 10-20 minutes.

**Note** Incubation time should be optimized for each cell line.

4. Remove the dye working solution and wash cells with GAP Junction Assay buffer.

**Note** For the adherent cells, detach cells from the plate using rubber policeman.

5. Resuspend cells in cell culture medium.

### Cell labelling protocol for DiD

1. Grow cells in cell culture medium in 6-well cell culture plates.
2. Remove the cell culture medium and add 0.5 mL of DiD working solution.
3. Incubate cells at 37 °C for 10-20 minutes.

**Note** Incubation time should be optimized for each cell line.

4. Remove the dye working solution and wash cells with GAP Junction Assay Buffer.

**Note** For the adherent cells, detach cells from the plate using rubber policeman.

5. Resuspend cells in cell culture medium.

### GAP junction assay

1. Mix Calcein stained cells and DiD labelled cells with 1:1 ratio and plate them in wells.

**Note** For the fluorescence microscopy, add 50 µL of each into the well of a 96-well plate and mix well.

**Note** For the flow cytometer, add 500 µL of each into the well of a 6-well plate and mix well.

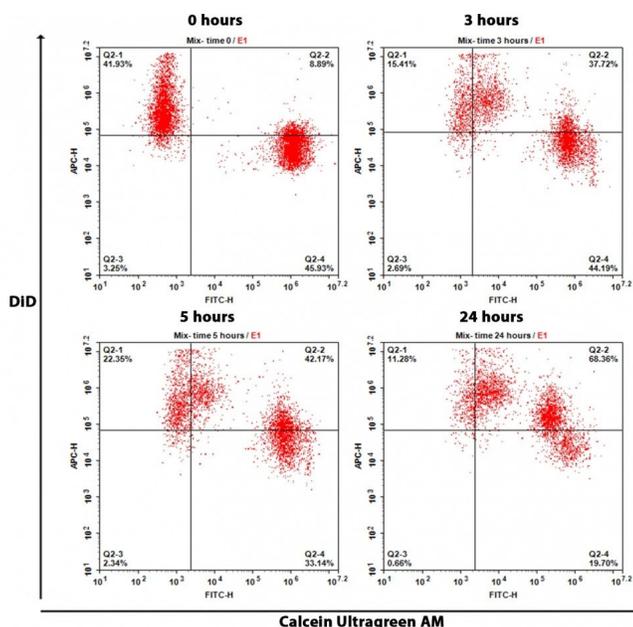
2. Incubate cells at 37 °C for 2-3 hours.
3. Monitor the cells with a fluorescence microscope using the FITC and Cy5 filter sets.
4. For flow cytometric analysis: For adherent cells, detach cells using rubber policeman. Once cells are in suspension or for cells in suspension, wash cells twice with DPBS or buffer of your choice. Resuspend cells in HHBS (cat# 20011), or DPBS or buffer of your choice and measure response with 530/30 nm filter (FITC channel) and 660/20 nm filter (APC channel).

were mixed well with 1:1 ratio and replated with cell culture medium. Response was measured using NovoCyte flow cytometer (ACEA Biosciences) with FITC and APC channel. As time progress, Q2-2 population (double positive population) increases.

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### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** The GAP junctions were analyzed by flow cytometry. HeLa cells were stained with Calcein Ultragreen AM and DiD separately as per the protocol. Cells