Cell Meter™ Cell Adhesion Assay Kit

Catalog number: 23010
Unit size: 100 Tests

Component	Storage	Amount
Component A: Calcein UltraGreen™ AM	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Adhesion Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)

OVERVIEW

The Cell Meter™ Cell Adhesion Assay Kit is a fast and sensitive assay for measuring cell-cell or cell-surface adhesion for a variety of cell types. In this assay, cells are labeled with Calcein UltraGreen AM and allowed to adhere. After removal of nonadherent cells, The fluorescence of Calcein UltraGreen is used to calculate the number of adherent cells. The use of our outstanding fluorogenic dye, Calcein UltraGreen AM provides a fast and sensitive method for measuring cell adhesion with a variety of cell types. Calcein UltraGreen AM is nonfluorescent but, once loaded into cells, is cleaved by endogenous esterases to produce highly fluorescent Calcein UltraGreen, a brightly fluorescent, pH-independent, cytoplasmic cell marker with the minimal interference to cell adhesion process. The Cell Meter™ cell adhesion assay is designed for use with fluorescence microplate readers. The robust performance of Calcein UltraGreen AM and simple procedure of the kit avoids problems associated with assays that utilize radioisotopes, which generate hazardous waste, and with assays that rely on the use of covalently coupled cell-surface labels, which can potentially alter cell function.

AT A GLANCE

Protocol summary

1. Add cells on a plate coated with desired coating material
2. Incubate cells at 37 °C to allow them to attach
3. Remove the unattached cells
4. Add Calcein Ultragreen AM working solution
5. Incubate the cells at 37 °C for 20-30 minutes
6. Remove supernatant and wash cells with HHBS or DPBS
7. Measure the fluorescence intensity using fluorescence microplate reader with Ex/Em = 490/525 nm

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

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
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.

Calcein Ultragreen AM stock solution
Add 50 µL of DMSO (Component C) into Calcein Ultragreen AM (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.

PREPARATION OF WORKING SOLUTION

Calcein Ultragreen AM working solution
Add 50 µL of Calcein Ultragreen AM stock solution into 10 mL of Adhesion Assay Buffer and mix well.

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

Note 10 mL Calcein Ultragreen AM working solution is enough for 100 tests.

SAMPLE EXPERIMENTAL PROTOCOL

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

1. Add 100 µL volumes of cells on a plate coated with desired coating material.
2. Incubate plate at 37 °C for 2 to 3 hours.

   Note For each cell line, optimal incubation time should be tested experimentally.
3. Remove the growth medium and unattached cells.
4. Add 100 µL of Calcein Ultragreen AM working solution and incubate plate at 37 °C for 20-30 minutes.

   Note For each cell line, optimal incubation time should be tested experimentally.
5. Remove the dye working solution and wash cells with 1X Hank's salt solution and 20 mM Hepes buffer or DPBS once.
6. Add 100 µL of Adhesion Assay Buffer to the wells.
7. Monitor the fluorescence intensity using a fluorescence microplate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

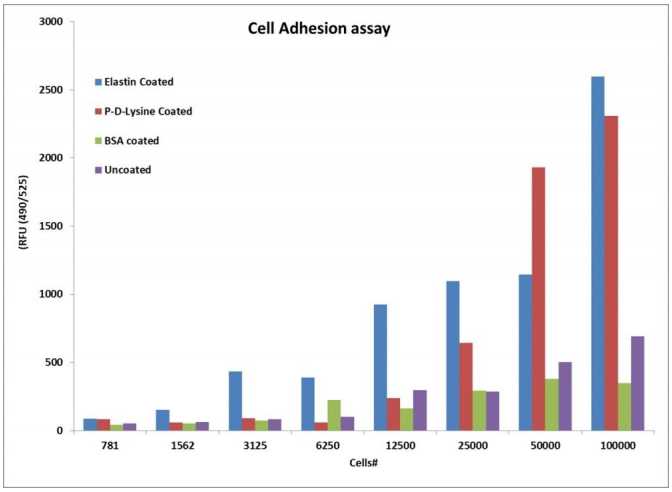

Figure 1. Cell adhesion measured with Cell Meter™ Cell Adhesion Assay Kit using a fluorescence microplate reader. Jurkat cells at different confluences or confluency levels were incubated in wells coated with different materials, and then stained with Calcein Ultragreen AM at 37°C for 30 mins. The signal was monitored at Ex/Em = 490/525 nm.

DISCLAIMER

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