Cystine Cellular Uptake Assay Kit

Catalog No.: abx298988

Size: 96 tests

Storage: Store the Cystine Analog, Fluorescence Probe, and Reductant in the dark at -20°C. Store the rest of the kit components at -20°C.

Application: For detection and quantification of Cystine Cellular Uptake activity in cell samples.

Introduction

Abbexa's Cystine Cellular Uptake Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Cystine Cellular Uptake activity. A Cystine analog is transported into cells in the same manner as normal Cystine. The Cystine analog reacts with fluorescent probes to emit detectable fluorescence, and from this the relative Cystine uptake can be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 28 ml
3. Cystine Analog: 0.5 ml
4. Fluorescence Probe: 0.3 ml
5. Reductant: 0.3 ml
6. Plate sealer: 2

Materials required but not provided

1. Microplate reader (Ex/Em 485 nm/535 nm)
2. PBS (0.01 M, pH 7.4)
3. Ethanol (anhydrous)
4. Trypsin
5. Culture medium
6. Pipette and pipette tips
7. 1.5 ml microcentrifuge tubes
8. Centrifuge
9. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Reagents

Bring all reagents to room temperature before use. Avoid repeated freeze-thaw cycles.

- **Cystine Analog Working Solution:** Prepare sufficient Cystine Analog Working Solution for the experiment. To create 500 µl of Cystine Analog Working Solution, mix 5 µl of Cystine Analog with 495 µl PBS (0.01 M, pH 7.4). The solution should be prepared fresh for use, and stored on ice in the dark until needed. Use within 2 hours once prepared.
- **Assay Working Solution:** Prepare sufficient Assay Working Solution for the experiment. To produce 1000 µl of Assay Working Solution, mix 5 µl of Fluorescence Probe,10 µl of Reductant, and 985 µl of Buffer Solution. The solution should be prepared fresh for use, and stored on ice in the dark until needed. Use within 2 hours once prepared.

2. Samples

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Cell samples:** Cells should be cultured and treated according to relevant culture protocols, the recommended cell density for assay is 6×10^5 cells per well.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

B. Assay Procedure

1. Seed 6×10^5 cells per well.
2. Add Trypsin to detach cells, then add culture media to neutralize.
3. Transfer the cell suspension to a microcentrifuge tube, split each cell suspension between a sample tube and a control tube.
4. Centrifuge at 300 × g for 5 minutes at 4°C.
5. Collect cells and wash 3 times with PBS (0.01 M, pH 7.4).
6. Preheat Cystine Analog Working Solution and PBS to 37°C for 5 minutes, then add 400 µl Cystine Analog Working Solution to sample tube.
7. Add 400 µl PBS (0.01 M, pH 7.4) to control tube.
8. Add 200 µl anhydrous ethanol to each tube and mix thoroughly, then centrifuge at 10,000 × g for 10 minutes. Collect the supernatant and store on ice for detection.
9. Add 50 µl of the sample supernatant to the sample wells.
10. Add 50 µl of the control supernatant to the control wells.
11. Add 200 µl of Assay Working Solution to all wells and mix thoroughly. Incubate at 37°C for 30 minutes.
12. Measure the fluorescence intensity at the excitation (485 nm) and emission (535 nm) wavelengths.

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C. Calculation of Results

The relative Cystine Cellular Uptake activity for samples can be derived with the following formula:

$$\text{Cellular Cystine Uptake} = F_{sample} - F_{control}$$

where:

F_{sample}	Fluorescence value of sample
$F_{control}$	Fluorescence value of control

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