Beta-Lite™ CCF-2 AM Lactamase Substrate *Cell-Permeable*

Catalog number: 12700
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 12700)
Beta-Lite™ CCF-2 AM Lactamase Substrate	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

Beta-Lite™ CCF-2 AM Lactamase Substrate *Cell-Permeable* is a live-cell, FRET-based substrate designed for sensitive and quantitative detection of β-lactamase activity in cell-based assays. The molecule is functionalized with acetoxymethyl (AM) ester groups to enable passive diffusion across the plasma membrane. Following intracellular uptake, endogenous esterases hydrolyze the AM esters, generating the negatively charged CCF-2 substrate that is retained within the cytosol for reliable intracellular signal detection. The intact substrate comprises a 7-hydroxycoumarin donor linked to a fluorescein acceptor through a β-lactam core. Upon violet light excitation, efficient intramolecular FRET produces green fluorescence. Cleavage of the β-lactam ring by β-lactamase disrupts FRET, resulting in a shift to blue emission. This ratiometric fluorescence change enables normalization for cell number and loading variability, supporting improved assay precision and reproducibility. Beta-Lite™ CCF-2 AM is ideally suited for β-lactamase reporter gene assays and other cell-based applications requiring sensitive, non-lytic detection of enzyme activity in viable cells.

AT A GLANCE

1. Plate cells as desired
2. Prepare and add Beta-Lite™ CCF-2 AM Lactamase substrate working solution to samples
3. Incubate samples at 37°C or RT for 30 to 60 minutes
4. Monitor the fluorescence intensity with fluorescence microplate reader or microscope using appropriate filter sets

Note: To ensure accurate results, allow substrate to reach room temperature before beginning the experiment.

KEY PARAMETERS

Fluorescence microscope

Emission	450 nm and 530 nm
Excitation	405 nm
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Cutoff	420 and 515 nm
Emission	450 nm and 530 nm
Excitation	405 nm
Recommended plate	Solid black Or Black wall/clear bottom plate

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

Prepare Beta-Lite™ CCF-2 AM Lactamase Substrate stock solution by adding 100 µL of DMSO into Beta-Lite™ CCF-2 AM Lactamase Substrate vial.

PREPARATION OF WORKING SOLUTION

Prepare working solution by adding 10 µL of Beta-Lite™ CCF-2 AM Lactamase substrate stock solution into 1 mL of HH Buffer (not provided, AAT Cat# 20011) with 0.04% PF-127 (not provided, AAT Cat# 20053). Protect the working solution from light by covering it with foil or placing it in dark.

Notes:

1. For best results, this solution should be used within 2 to 3 hours of its preparation.
2. 1 mL of working solution is enough for 10 tests.
3. If your cells contain organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in well concentration should be 0.5-2 mM) to reduce leakage of the de-esterified indicators. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

Following is our recommended protocol for loading AM esters into live cells. This protocol only provides a guideline and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.
2. Next day, remove the cell culture medium and wash once with HH buffer.
3. Add 100 µL of Beta-Lite™ CCF-2 AM Lactamase Substrate working solution.
4. Cover the plate and protect it from light and evaporation.
5. Incubate the substrate-loaded plate in a cell incubator at 37°C or RT for 60 to 120 minutes.
   Note: For best results, incubation time and temperature must be optimized for each cell lines tested.
6. Read the plate with appropriate filter sets using fluorescence microscope or microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

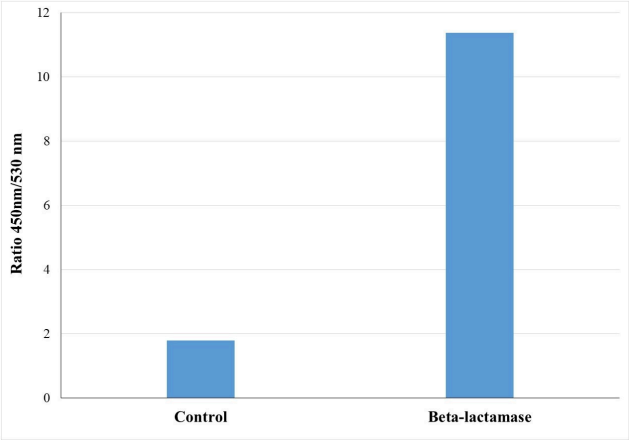

Figure 1. Beta lactamase activity measured using Beta-Lite™ CCF-2 AM Lactamase Substrate (#12700) in Jurkat cells. Jurkat cells were loaded with Beta-Lite™ CCF-2 AM Lactamase Substrate followed by lysis using mild detergent. 1 mU/mL of Beta-lactamase enzyme was added to activate the substrate. Control sample had no enzyme added. Fluorescence was measured after one hour of incubation and emission measured at 450 nm and 530 nm with Clariostar microplate reader (BMG Labtech) and ratio was calculated.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.