Cell Meter™ Live Cell ATP Assay Kit

Catalog number: 23015
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 23015)
Component A: ATP Red™	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (50 mL)
Component C: DMSO	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Adenosine triphosphate (ATP) plays a fundamental role in cellular energetics, metabolic regulation and cellular signaling. It is referred as the "molecular unit of currency" of intracellular energy transfer to drive many biological processes and chemical synthesis in living cells. ATP also serves as a signaling molecule for cell communication and plays an important role in DNA and RNA synthesis. It is localized in mitochondria, where cellular respiration occurs. ATP levels can be used to measure cell proliferation and cell cycle dynamics. AAT Bioquest offers a variety of bioluminescence, fluorescence and colorimetric assay kits to determine ATP level in solutions. Cell Meter™ Live Cell ATP Assay Kit enables researchers to monitor ATP levels in live cells using ATP Red™, a cell-permeable red fluorescent imaging probe for detecting ATP. ATP Red™ is designed to monitor ATP concentrations in the mitochondria of living cells. The probe has minimal cross reactivity to AMP, ADP, CMP, CDP, CTP, UMP, UDP, UTP, GMP, GDP or GTP.

AT A GLANCE

Protocol summary

1. Prepare cells in a growth medium.
2. Incubate the cells with the ATP Red™ working solution at 37°C for 15 to 30 minutes.
3. Remove the ATP Red™ working solution.
4. Analyze with a fluorescence microscope using a Cy3/TRITC filter set.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy3/TRITC filter set
Excitation	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For Adherent Cells

1. Plate cells overnight in a growth medium. For a 96-well plate, use 10,000 to 40,000 cells/well/90 µL. For a 384-well plate, use 2,500 to 10,000 cells/well/20 µL.

For non-adherent cells

1. Centrifuge the cells from the culture medium. Suspend the cell pellets in culture medium, preparing 50,000-100,000 cells/well/90 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/20 µL for a 384-well poly-D lysine plate. Before starting your experiment, centrifuge the plate at 800 rpm for 2 minutes with the brake off.

   Note: Each cell line should be individually assessed to determine the optimal cell density.

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

ATP Red™ Stock Solution (200X)

1. Add 50 µL of DMSO (Component C) to the vial containing ATP Red™ (Component A) to prepare a 200X stock solution.

   Note: 50 µL of the 200X ATP Red™ stock solution is sufficient for one 96-well plate. Any unused ATP Red™ stock solution can be aliquoted and stored at ≤ -20°C, protected from light. Avoid freeze/thaw cycles.

PREPARATION OF WORKING SOLUTION

ATP Red™ Working Solution

1. Add 5 µL of the 200X stock solution (Component A) to 1 mL of cell culture medium and mix thoroughly.

   Note: The ATP Red™ probe is suitable for use with the cell culture media of most cell lines we have tested. Staining conditions can be adjusted based on the specific cell type.

SAMPLE EXPERIMENTAL PROTOCOL

Stain cells

1. Prepare cells in a growth medium.
2. Add an equal volume of ATP Red™ working solution to each well in the cell plate. Use 100 µL per well for a 96-well plate or 25 µL per well for a 384-well plate.

   Note: The ideal concentration of ATP Red™ depends on the specific application.

3. Incubate the cells at 37°C for 15-30 minutes, protected from light.
4. Remove the working solution from each well. Then, wash the cells twice with Assay Buffer (Component B) or any other buffer of your choice.
5. Use a fluorescence microscope with a Cy3/TRITC filter set to observe the fluorescence signal in the cells

EXAMPLE DATA ANALYSIS AND FIGURES

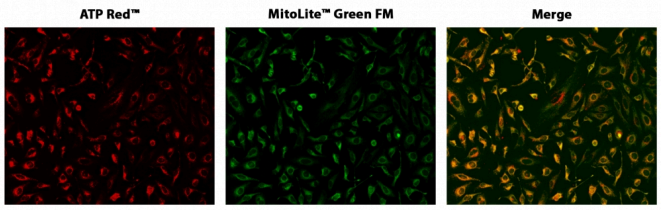

Figure 1. The fluorescence images of HeLa cells co-stained with ATP Red™ and MitoLite™ Green FM in a 96-well black-wall clear-bottom plate. HeLa cells were stained with ATP Red™ for 15 min and then incubated with 100 nM MitoLite™ Green FM (Cat#22695) for another 30 minutes. Washed twice with assay buffer before imaging. ATP Red™ and MitoLite ATP Red™ signals overlay well. (Far right image) The cells were imaged using a fluorescence microscope with a Cy3/TRITC and FITC filters.

DISCLAIMER

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