Cell Meter™ Live Cell ATP Assay Kit

Catalog number: 23015
Unit size: 100 Tests

Component	Storage	Amount
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 growth medium
2. Incubate cells with ATP Red™ working solution at 37 °C for 15-30 minutes
3. Remove ATP Red™ working solution
4. Analyze with a fluorescence microscope using a Cy3/TRITC filter set

KEY PARAMETERS

Fluorescence microscope

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

CELL PREPARATION

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

For non-adherent cells
Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 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. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.

Note Each cell line should be evaluated on an individual basis 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 (500X)

Add 50 µL of DMSO (Component C) into the vial of ATP Red™ (Component A) to make a 500X stock solution.

Note 50 µL of the 500X ATP Red™ stock solution is enough for one 96-well plate. The unused ATP Red™ stock solution can be aliquoted and stored at ≤ -20 °C in smaller aliquots. Protect from light and avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

ATP Red™ working solution
Add 5 µL of the 500X stock solution (Component A) into 1 mL cell culture medium, and mix well.

Note The ATP Red™ probe is compatible with cell culture media of most cell lines we tested. The staining conditions may be modified according to the particular cell type.

SAMPLE EXPERIMENTAL PROTOCOL

Stain cells

1. Prepare cells in growth medium.
2. Add equal volume [100 µL/well (96-well plate) or 25 µL/well (384-well plate)] of ATP Red™ working solution in the cell plate.

   Note The optimal concentration of ATP Red™ varies depending on the specific application.
3. Incubate the cells at 37 °C for 15-30 minutes, protected from light.
4. Remove working solution from each well. Wash cells with Assay Buffer (Component B) or buffer of your choice twice.
5. Observe the fluorescence signal in cells using a fluorescence microscope with a Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

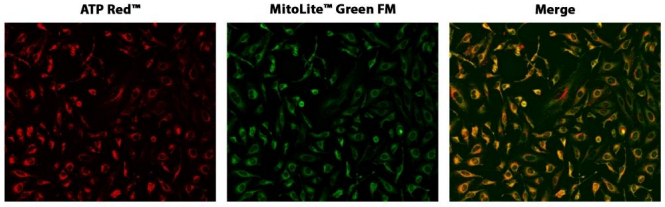

Figure 1. The fluorescence images of HeLa cells co-stained with ATP Red™ and MitoLite™ Green FM in a 96-well black-wall clear-bottom plate. HeLa cells were stained with ATP Red™ for 15 min and then incubated with 100 nM MitoLite™ Green FM (Cat#22695) for another 30 minutes. Washed twice with assay buffer 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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