

Cellular ATP Kit HTS

Measurement of ATP in cell cultures with just one reagent addition



- Stable light: Half-life >1.5 h
- Fast assay: Results within minutes
- Sensitive: 10^{-15} mol ATP (single cells detected)
- User-friendly: Mix & measure
- Reliable: ATP Standard in liquid form

Leader in luminescent
ATP-assays

BioThema
LUMINESCENT ASSAYS

The BioThema logo features the company name in a bold, blue, sans-serif font. To the right of the name is a stylized graphic of a yellow test tube with a grey shadow, set against a white background that resembles a folded piece of paper.

Cellular ATP Kit HTS

Intended use

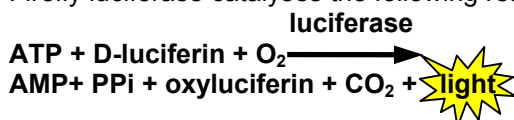
Cellular ATP Kit HTS is intended for enumeration of viable cells by quantification of cellular ATP (adenosine triphosphate). The assay is intended for rapid analysis of proliferation and cytotoxicity of mammalian cells. The assay of cellular ATP replaces methods such as tritiated thymidine incorporation and tetrazolium salt reduction. The Cellular ATP Kit HTS is especially designed for High Throughput Screening (HTS); enabling assay in microplates with manual or fully automated handling. The assay format is a mix and measure addition of ATP Reagent containing cell lysing agent, ATPase inhibitors and stabilizers for the firefly luciferase enzymatic reaction. The $T_{1/2}$ of the light emission is 3 - 12.5 hours depending on culture medium and presence of fetal calf serum, enabling convenient screening of large numbers of samples. The Cellular ATP Kit HTS is useful for quantification of ATP in range of 10^{-11} - 10^{-7} mol/L. This dynamic range covers all commonly used seeding cell number and culture media.

Applications

1. Drug discovery
2. Toxicity testing
3. Hormone effects
4. Tumour chemosensitivity assay
5. Cell proliferation

Assay principles

Firefly luciferase catalyses the following reaction:



The assay has been optimised to give a stable light at all ATP levels up to 10^{-6} mol/L. The reagent contains D-luciferin, magnesium ions, PPi (inorganic pyrophosphate), BSA (bovine serum albumin) and an ATPase inhibitor to reduce ATP consumption by ATPases present in e.g. fetal calf serum.

Instruments

The stable light makes it possible to manually add the ATP Reagent and the ATP Standard with a multi-channel pipette or to use a microplate luminometer with automatic reagent dispensers.

Kit contents

The kit contains reagents for 1200 assays (each vial of ATP Reagent SL is sufficient for 240 assays in a microplate luminometer).

1. ATP Reagent SL (5 vials). Lyophilised reagent containing D-luciferin, luciferase and stabilizers
2. Lysing Diluent, 12 mL (5 vials). Buffer used to reconstitute ATP Reagent SL containing lysing agent and ATPase inhibitor
3. ATP standard 5 mL (5 vials; 10^{-5} mol/L ATP)

Assay procedure using internal ATP Standard

In this procedure a known amount of ATP is added in the assay of each individual sample. This strongly increases the reliability of the assay and makes it possible to express ATP results in moles rather than rlu or other non-chemical units. The assay can also be performed using external ATP Standard. However, this is a less reliable procedure if the sample composition varies.

Microplate luminometer with two dispensers:

Culture cells in 100 μ L culture media in microplate wells. Make sure to have controls of complete media and additionally added agents.

1. Add 50 μ L ATP Reagent SL with dispenser 1 and measure the light emission corresponding to sample ATP, I_{smp}
2. Add 10 μ L of ATP Standard with dispenser 2 and measure the light emission corresponding to sample plus standard ATP, $I_{\text{smp+std}}$

Calculations:

Calculate amount of ATP (moles) in the sample by the equation:

$$\text{ATP}_{\text{smp}} = 10^{-10} \times I_{\text{smp}} / (I_{\text{smp+std}} - I_{\text{smp}})$$

The factor 10^{-10} is the amount (moles) of ATP Standard in the well (10 μ L of 1×10^{-5} mol/L).

Product characteristics

Sensitivity: 10^{-15} mol ATP

No. of determinations (microplate): 1200

Ordering info

Article No: 155-050

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