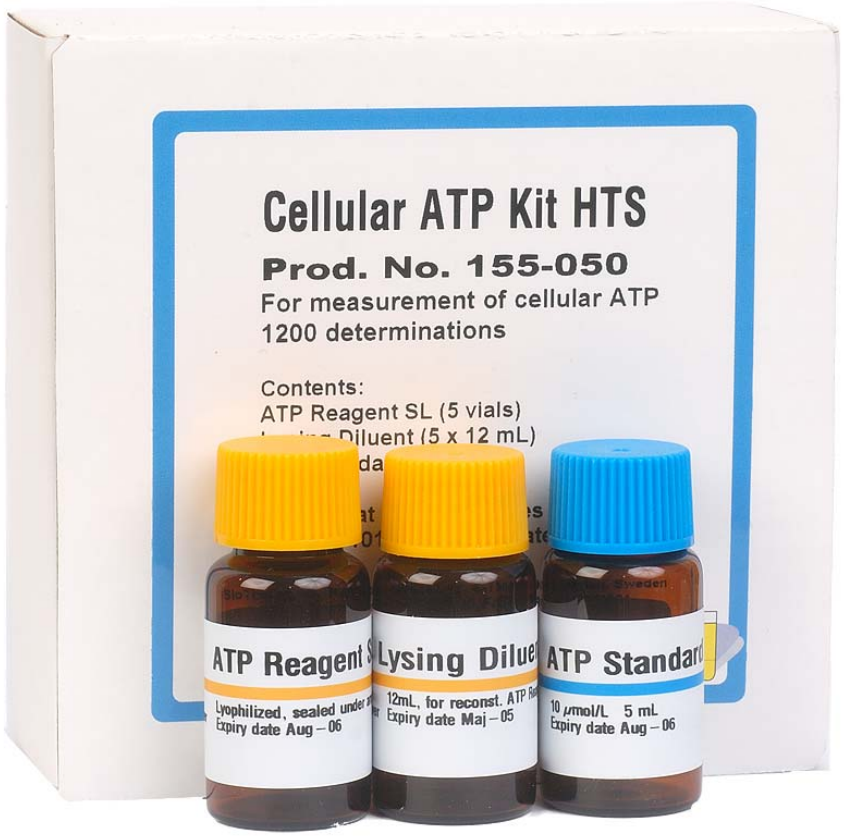
Cellular ATP Kit HTS

Measurement of ATP in cell cultures with just one reagent addition



- Fast Assay: Results within minutes
- Sensitive: 10^{-15} mol ATP (single cells detected)
- User-friendly: Mix & measure
- Reliable: Stable, Ready-to-use ATP Standard
- Cost-efficient: No Standard curve required

Leader in luminescent ATP-assays

BioThema
LUMINESCENT ASSAYS

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 testing
5. Cell proliferation

Assay principles
Firefly luciferase catalyses the following reaction:

$$\text{ATP + D-luciferin + } O_2 \xrightarrow{\text{luciferase}} \text{AMP+ PPi + oxyluciferin + } CO_2 + \text{light}$$

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 stabilisers
2. Lysing Diluent, 12 mL (5 vials). Buffer used to reconstitute ATP Reagent SL containing lysing agent and an 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 µL culture media in microplate wells. Make sure to have controls of complete media and additionally added agents.
1. Add 50 µL ATP Reagent SL with dispenser 1 and measure the light emission corresponding to sample ATP, I_{smp}
2. Add 10 µL of ATP Standard with dispenser 2 and measure the light emission corresponding to sample plus standard ATP, $I_{smp+std}$

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

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

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

Product characteristics
Sensitivity: 10^{-15} mol ATP
No. of determinations (microplate): 1200

Ordering info

Art No	*Description*
155-050	Cellular ATP Kit HTS

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Cellular ATP Kit HTS Prod. No. 155-050

Instruction for use

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 cytotoxicity and proliferation in cell studies. The assay of cellular ATP replaces methods such as tritiated thymidine incorporation and tetrazolium salt reduction. If a stable light emission is not obtained, the ATPases are not completely inhibited. We then recommend the Cell Viability Kit SL containing a strongly ATPase inactivating agent. The Cellular ATP Kit HTS is especially designed for HTS screening methodology; enabling assay in 96 well format with manual or fully automated handling. The assay format is a mix and measure addition of ATP Reagent containing cellular lysis agent and stabilizers for the firefly luciferase enzymatic reaction. The $T_{1/2}$ of the light emission is 3 hours in medium containing 10% FCS (Fetal Calf Serum) and 12.5 hours in media supplemented with e.g. vitamin mixture F12, enabling screening of a large number 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 the commonly used seeding cell number and culturing media.

Assay principles

Firefly luciferase catalyses the following reaction:

$$\text{ATP} + \text{D-luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{AMP} + \text{PPi} + \text{oxyluciferin} + CO_2 + \text{light}$$

The assay has been optimised to give a stable light at all ATP levels up to 10^{-7} 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 medium supplement.

Kit contents

1. ATP Reagent SL. 5 vials of lyophilised reagent containing D-luciferin, luciferase and stabilizers.
2. Lysing Diluent 12 mL. 5 vials containing Tris(hydroxymethyl) aminomethane, EDTA buffer adjusted to pH 7.75 with acetic acid, lysing agent and an ATPase inhibitor.
3. ATP Standard 5 mL. 5 vials containing 10^{-5} moles/L of ATP.

The kit should be stored at +4 °C. However, during normal transport times the kit is not affected by ambient temperature. The kit and the individual components are labelled with expiry date assuming storage at +4 °C.

Reagent reconstitution

The ATP Reagent SL is reconstituted by adding the entire content of the Lysing Diluent vial (12 mL) to the reagent vial. It is very important to avoid contamination. Please use a clean pair of tweezers to remove the rubber stopper from the ATP Reagent vial. Pour the diluent gently into the reagent vial to dissolve the lyophilised reagent. Subsequently pour the solution back into the Lysing Diluent vial. Once reconstituted the reagent must be protected from light. In reconstituted ATP Reagent SL the luciferase activity slowly decays, e.g. storage at +4 °C for 4 days decreases activity of the ATP Reagent SL by 15%. However, by calibrating the assays with ATP Standard makes it possible to use the ATP Reagent SL for several days at +4 °C or for 1 month at -18 °C. Reagents should be allowed to attain room temperature before the assay (the optimum temperature for the luciferase reaction is around 25 °C.

Instrumentation

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 dispensers.

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 an external ATP Standard (cf. below). However, this is a less reliable procedure in most cases. The use of ATP Standard as described below compensates for variation of reaction conditions affecting the luciferase activity and for instrument and microplate variations. The fact that different media give different light emission with the same amount of ATP is therefore not a problem.

A Microplate luminometer with injectors and shaking

1. Culture cells in 100 µL culture media in white clear bottom wells. Make sure to have controls of complete media and any additionally added agents.
2. Dispense 50 µL ATP Reagent SL with dispenser 1 to all wells. Shake.
3. Measure the light emission from all wells corresponding to sample ATP, I_{smp}, using an integrated 5 second reading.
4. Dispense 10 µL of ATP Standard with dispenser 2 to all wells. Shake.
5. Measure the light emission corresponding to sample plus standard ATP, $I_{smp+std}$, taking another 5 second reading.
6. Calculate the amount of sample ATP in the well by the following equation:

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

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

B Microplate luminometer with manual pipetting

1. Culture cells according to A 1.
2. Dispense 50 µL ATP Reagent SL into all wells using a multichannel pipette. Shake.
3. Measure the light emission according to A 3.
4. Dispense 10 µL of ATP Standard into all wells using a multi-channel pipette. Shake.
5. Measure the light emission corresponding to sample plus standard ATP according to A 5.
6. Calculate the amount of sample ATP in the well using the same formula as in A 6.

Assay procedure using external ATP Standard

Add 10 µL ATP Standard to 0.99 mL complete medium, mix and add 100 µL of the mixture to 4-8 empty wells. Other wells contain cultured cells in 100 µL medium. Omit steps 4-6 in the procedures described above. Please note that FCS contains ATPases, which will rapidly reduce the amount of ATP Standard prior addition of the ATP Reagent containing the ATPase inhibitor. The average light emission from the wells containing ATP Standard, I_{std}, is used in the equation:

$$ATP_{smp} = 10^{-11} \times I_{smp} / I_{std}$$

The factor 10^{-11} is the amount (moles) of ATP Standard in the well (100 µL of 1 x 10^{-7} mol/L).

Procedural notes

1. The number of seeded cells should be chosen to give an ATP level in the well in the interval 10^{-14} - 10^{-10} moles (lower limit somewhat dependent on luminometer sensitivity). For example 100-10 000 cells gives an ATP level (depends of cell type) in the interval of 10^{-13} - 10^{-11} moles. With the internal standard procedure, high sample ATP levels approaching the concentration obtained from the ATP Standard tend to give too low values on ($I_{smp+std} - I_{smp}$) resulting in an overestimation of ATP_{smp}. By using the internal ATP Standard light inhibition due to proliferating and cytotoxic agents is compensated for.

2. The ATP Standard volume should be as low as possible with a retained precision. Ideally the volume should be <1% of the total reaction volume, since the dilution effect coming from the addition of ATP Standard otherwise can't be neglected. With a total reaction volume of 150 µL an ATP Standard volume of 10 µL is somewhat too high, but has to be accepted since lower volumes of ATP Standard can't normally be added with high precision.

3. The light emission should be measured as soon as possible after addition of reagents (ATP Reagent and ATP Standard, resp.). Maximum light emission is normally obtained within a few seconds and will then start to decay. The light decays between 0.1- 0.7 %/min depending on the culturing media used. Whenever changing to new media or new types of cells confirm that decay rate is acceptable by measuring the plate once more e.g. 10 min after the first measurement and calculating the decay rate.

4. Every series of assays should include at least two blanks in the beginning and at the end. Blanks should contain all supplements to medium (e.g. specific proliferation agents) and should be assayed and calculated exactly as normal samples containing cells. Blanks calculated by the formula above are subtracted from sample ATP values calculated in the same way.