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# Cell Viability Kit SL Prod. No. 188-441

Instructions for use

## Intended use

Cell Viability Kit SL is intended for cell proliferation and cytotoxicity studies by determination of total ATP (adenosine triphosphate). Extractant B/S included in the kit releases ATP from most types of cells and inactivates ATP degrading enzymes. For maximum accuracy, the level of extracellular ATP should be low compared to the intracellular level. With low numbers of cells, ATP degrading enzymes are not a problem and 155-050 Cellular ATP Kit HTS can be used for mammalian but not microbial cells. This kit allows a mix-and-measure assay with only one step (the Cell Viability Kit SL requires two steps – first extraction and then light measurement). If a higher sensitivity is required or with bacterial samples, the 266-311 ATP Biomass Kit HS is recommended. If extracellular ATP levels are not negligible the 266-111 Intracellular ATP Kit HS can be used. Assays of ATP replaces methods such as tritiated thymidine incorporation and tetrazolium salt reduction.

The Cell Viability Kit SL 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.

All living cells contain ATP where it plays the role of energy currency between different cellular processes. When cells die of natural causes, ATP is normally degraded. The intracellular concentration of ATP is carefully regulated to similar levels in all types of cells. ATP is therefore a good estimate of the total intracellular volume. Most bacterial cells contain approx. $2x10^{-18}$ mol ATP per cell, while most eukaryotic cells, as a result of their larger size, contain $10^{-15}$ mol ATP or more.

## Assay principles

Before the assay, ATP is released from the cell using Extractant B/S. ATP is assayed using ATP Reagent SL.

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

The intensity of the light is proportional to the amount of ATP and is measured in a luminometer.
For maximum accuracy, the light emission can be measured before and after addition of a known amount of ATP standard. This makes it possible to calculate the amount of ATP in unknown samples expressing the result in pmol ($10^{-12}$ mol).

## Instruments

Any tube or microplate luminometer. It should be noted that most tube luminometers are about one order of magnitude more sensitive. If a microplate luminometer is used it is an advantage if it has dispensers (ideally three dispensers) and is able to shake the microplate for good mixing.

## Kit contents

The kit contains reagents for 320 assays in cuvette format or 1600 assays in 96 well microplate format (each vial of ATP Reagent SL is sufficient for 200 assays in a microplate luminometer).

1. ATP Reagent SL (8 vials). Lyophilised reagent containing luciferase and luciferin.
2. Diluent B 16 mL (8 vials). Buffer used to reconstitute ATP Reagent SL.
3. Extractant B/S 10 mL (4 vials).
4. ATP standard 5 mL (4 vials; $10^{-5}$ mol/L ATP).

## Reagent Reconstitution

Remove and discard screw lid and rubber stopper from ATP Reagent SL. ATP contamination from hands is avoided by using a clean pair of pincers when removing the rubber stopper. Remove screw lid from vial containing Diluent B. Pour approx. half of the content of Diluent B into the ATP Reagent SL vial and swirl the content to mix. Thereafter pour the liquid back again into the Diluent vial and swirl to mix. Store the reagent in the refrigerator covered from light.
Extractant B/S and ATP Standard are ready to use.

## Applications

This kit can be used for determination of ATP levels in e.g. animal or bacterial cells in a variety of circumstances:

1 Estimation of cell numbers
2 Cell proliferation studies
3 Cytotoxicity studies

**Assay procedure**

Tube luminometers

This procedure can be used for most types of cell suspensions including bacterial samples (provided the sample volume is 50 µL or less; cf. Procedural Note 6)

1 Add 50 µL Extractant B/S to the cuvette and mix.
2 Add 400 µL reconstituted ATP Reagent SL and mix.
3 Measure light emission, $I_{smp}$.
4 Add 10 µL 10 µmol/L ATP Standard, i. e. 100 pmol ATP, and mix.
5 Measure light emission, $I_{smp+std}$.

With tube luminometers it is advantageous to perform all steps for one sample before starting with the next sample (no cross-talk between cuvettes; cf. Procedural Note 2).

Microplate luminometers

The procedure below is designed for a sample volume of 100 µL (animal cells) or 20 µL (bacterial cells; cf. Procedural Note 6). The procedure with a luminometer for 96 well microplates (cf. Procedural Note 2) is as follows:

1 Add 20 µL Extractant B/S to all wells and mix.
2 Add 80 µL reconstituted ATP Reagent SL to all wells and mix.
3 Measure light emission, $I_{smp}$, from all wells
4 Add 10 µL 10 µmol/L ATP Standard, i.e. 100 pmol ATP, to all wells and mix.
5 Measure light emission, $I_{smp+std}$, from all wells.

If the luminometer has dispensers they should be used for the additions in steps 1, 2 and 4. Higher sample volumes than 100 µL may result in splashing with some microplates and luminometers. If the luminometer does not shake the microplate, it may be necessary to take repeated readings until maximum light emission is attained. If the luminometer does not have dispensers the procedure is in principle the same, i.e. each step is completed for the entire microplate before the next step is started. With manual additions of reagents one should work as rapidly as possible, since the light emission goes down by approx. 0.5% per minute. When trying out the procedure read the plate several times with a few minutes in between to confirm that the light emission has a maximum decrease of 1% per minute. When trying out alternative volumes, please observe Procedural notes 6-9 or contact BioThema for support.

**Calculations:**

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

$$ATP_{smp} = 100 \times I_{smp} / ( I_{smp+std} - I_{smp} )$$

The factor 100 is the amount of ATP Standard added (100 pmol).

**Procedural notes**

1 Cuvettes, microplates and pipette tips must be ATP free. Use disposable plastics stored well covered only. Autoclaving is a way to release ATP from cells but not to remove it. Use powder-free gloves for your hands and handle e.g. cuvettes with a clean pair of pincers.
2 White or black microplates can be used. Black microplates show much less cross-talk between wells (important if wells contain widely different cell numbers). Use white microplates if cell numbers are very low.
3 Some plastics show a delayed fluorescence after exposure to light. Luciferin is rapidly destroyed in bright light. Avoid bright light in the laboratory, while working with ATP assays.
4 Run blanks containing the medium in which the cells are suspended.
5 Always perform at least duplicate assays, since ATP contamination in cuvettes, microplates or on pipette tips may jeopardize the results.
6 With bacterial samples the ratio between the volumes of sample and Extractant B/S should never exceed 1/1 (it may however be lower).
7 The ratio between volumes of Extractant B/S and ATP Reagent SL should never exceed 1/4 (it may however be lower, e.g.1/8).
8 The volume of ATP Standard should always be 10 µL.
9 For a particular final reaction mixture containing sample and reagents the light signal increases with the volume of the mixture. Decreasing the volumes should be avoided if maximum sensitivity and accuracy is required.
10 The $(I_{smp+std} - I_{smp})$ value should be at least 10x higher than the $I_{smp}$ value. If not, the sample should be diluted.
11 If the same culture medium is used in all samples, it is possible to perform the assay without internal ATP standardisation of every assay, i. e. steps 4 and 5 in the procedures described above can be omitted. Instead the medium is assayed with and without ATP Standard and the $(I_{smp+std} - I_{smp})$ value is used for all samples.
12 All reagents are available as separate items.

**References**

[1] Lundin, A. (2000) Use of firefly luciferase in ATP related assays of biomass, enzymes and metabolites. Methods Enzymol. **305**, 346-370.