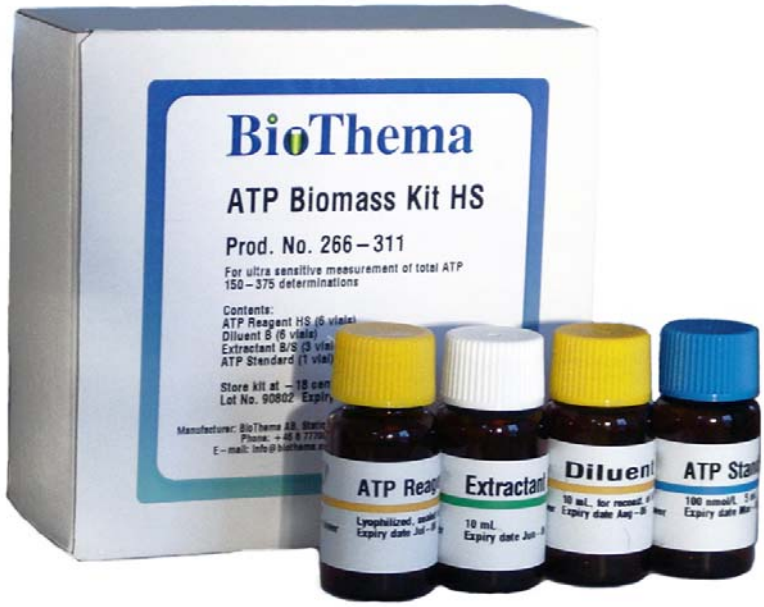
ATP Biomass Kit HS

Measurement of total ATP (extra- plus intracellular) in virtually all types of samples



- Fast assay: Results within minutes
- Detection limit: 10^{-17} mol ATP (~ 5 bacteria)
- Reliable: ATP standard in liquid form
- Flexible: Choice of µ-plate & cuvette methods
- Efficient extractant: Animal & microbial cells

Leader in luminescent ATP-assays

BioThema
LUMINESCENT ASSAYS

ATP Biomass Kit HS

Intended use
This kit is intended for determination of total ATP (extra- plus intracellular) in samples containing living cells. For determination of intracellular ATP one should consider using 266-111 Intracellular ATP Kit HS or 266-112 Microbial ATP Kit HS. For cell proliferation/cytotoxicity studies please, consider 155-051 Cellular ATP Kit HTS. All living cells contain ATP where it plays the role of energy currency between different cellular processes. The intra-cellular concentration of ATP is carefully regulated to similar levels in all types of cells. ATP is there-fore 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. The detection limit of the reagent is $10x10^{-18}$ mol ATP with a sensitive luminometer.

Applications
The sensitivity of this kit allows determination of low ATP levels in a variety of circumstances provided extracellular ATP is not a problem:
1. Bacteriological control of liquids (e. g. drinking water, process water, beverages and beer)
2. Pharmaceuticals and cosmetics
3. Cell adhesion to surfaces
4. Quality assurance of laundry
5. Bacterial growth
6. Antibiotic effects on bacteria

Assay principles
Before the assay, ATP is released from the cell using Extractant B/S. ATP is assayed using ATP reagent HS.

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

The intensity of the light is proportional to the amount of ATP and is measured in a luminometer. The light emission is 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 can be used. It should be noted that tube luminometers are about one order of magnitude more sensitive. If a microplate luminometer is used it should have minimum two dispensers and should be able to mix the microplate.

Kit contents
The kit contains reagents for 150-375 assays (each vial of ATP Reagent HS is sufficient for 25 assays in a tube luminometer and 62.5 assays in a microplate luminometer).
1. ATP Reagent HS (6 vials). Lyophilised reagent containing luciferase and luciferin. The luciferase activity in the reconstituted reagent consumes ATP at a rate of approx. 6%/min.
2. Diluent B 10 mL (6 vials). Buffer used to reconstitute ATP Reagent HS.
3. Extractant B/S 10 mL (3 vials).
4. ATP standard 5 mL (1 vial; 10^{-7} mol/L ATP).

Assay procedure using internal ATP Standard
1 Add 20-50 µL Extractant B/S to a cuvette.
2 Add 20-50 µL sample to the cuvette.
3 Add 160-400 µL reconstituted ATP Reagent HS and measure light emission, I_{smp}.
4 Add 10 µL 10^{-7} mol/L ATP Standard, i. e. 1 pmol ATP, and measure light emission, $I_{smp+std}$.

Calculations:
Calculate amount of ATP (pmol) in the sample by the equation:
$ATP_{smp} = I_{smp} / (I_{smp + std} - I_{smp})$

Product characteristics
Sensitivity: 10^{-17} mol ATP
ATP consumption (decay rate of light): 6 %/min
No. of determinations (tubes): 150
No. of determinations (microplate): 375

Ordering info
Article No: 266-311

Cellular ATP Kit, Nov. 2004

BioThema AB
Stationsvägen 17
SE-136 40 Haninge
Sweden
Web: www.biothema.com

BioThema LUMINESCENT ASSAYS

Phone: +46 (0)8 777 00 20
Fax: +46 (0)8 777 00 21
E-mail: info@biothema.com

BioThema AB
S-136 40 Handen
Sweden
VAT No. SE556303345401

Phone **+46 (0)8 7770020**
Fax **+46 (0)8 7770021**
E-mail: **info@biothema.com**
Website: **www.biothema.com**

ATP Biomass Kit HS Prod. No. 266-311

Instructions for use

Intended use

This kit is intended for determination of total ATP (adenosine triphosphate) in samples containing living cells. If you are not certain that the ATP Biomass Kit HS is the right one for your application, please check the Product Selection Guide at www.biothema.com (click Products/Kits/Product selection guide).

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.

The luciferase activity in ATP Reagent HS is so high that if contaminated during reconstitution it will degrade the ATP background by one order of magnitude every 38 min. After mixing sample and ATP Reagent HS the light intensity decays by approx. 6% per min. This is slow enough to allow manual mixing and a tube luminometer without reagent dispenser can be used. The detection limit of the reagent is $10x10^{-18}$ mol ATP with a sensitive luminometer. This corresponds to 5 bacterial cells. We must here differentiate between bacterial cells and CFU, since one CFU may consist of several cells. If the detection limit is not adequate, it is possible to concentrate the sample by microfiltration, a procedure that will also remove most of the extracellular ATP.

Assay principles

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

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

The intensity of the light is proportional to the amount of ATP and is measured in a luminometer. The light emission is 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 tube luminometers are about one order of magnitude more sensitive. If a microplate luminometer is used it should have minimum two dispensers and should be able to shake the microplate.

Kit contents

The kit contains reagents for 150-375 assays (each vial of ATP Reagent HS is sufficient for 25 assays in a tube luminometer and 62.5 assays in a microplate luminometer).

1. ATP Reagent HS (6 vials). Lyophilised reagent containing luciferase and luciferin.
2. Diluent B 10 mL (6 vials). Buffer used to reconstitute ATP Reagent HS.
3. Extractant B/S 10 mL (3 vials).
4. ATP standard 5 mL (1 vial; 100 nmol/L ATP).

Reagent Reconstitution

Extractant B/S and ATP Standard are ready to use. Remove and discard screw lid and rubber stopper from ATP Reagent HS. 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. The content of Diluent B is poured into the ATP Reagent HS vial and then back again into the Diluent vial. Store the reagent in the refrigerator covered from light. The activity may go down to half the initial activity during one week. However, correct ATP results will be obtained, if assays are calibrated with internal ATP standard.

Applications

The sensitivity of this kit allows determination of low ATP levels in a variety of circumstances:

1 Liquids (e. g. drinking water, process water, environmental samples)
2 Cell adhesion to surfaces (e. g. biocompatibility studies)
3 Quality assurance of laundry
4 Bacterial growth.
5 Antibiotic effects on bacteria

Assay procedure

Pre-treatment of sample:

When using ATP to estimate bacterial biomass, the bacteria must be the only source of ATP. Non-bacterial ATP may be degraded using ATP Eliminating Reagent coming with Intracellular ATP Kit HS or Microbial ATP Kit HS (cf. Product selection guide at www.biothema.com).

Concentration of cells:

If the bacterial levels are low, samples may be concentrated by microfiltration. Please consult BioThema for details.

ATP assay with tube luminometer:

1 Add 50 µL Extractant B/S to a cuvette.
2 Add 50 µL sample to the cuvette.
3 Add 400 µL reconstituted ATP Reagent HS and measure light emission, I_{smp}.
4 Add 10 µL 10^{-7} mol/L ATP Standard, i. e. 1 pmol ATP, and measure light emission, $I_{smp+std}$.
5 Calculate amount of ATP (pmol) in the sample by the equation:

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

ATP assay with microplate luminometer:

The sensitivity with microplate luminometers is about one order of magnitude lower than what can be achieved with tube luminometers. The lower sensitivity with microplate luminometers comes largely from the lower assay volume (less light is created). The procedure with a microplate luminometer with 3 reagent dispensers would be as follows:

1 Add 20 µL sample to each well.
2 Inject 20 µL Extractant B/S with dispenser 1 followed by mixing.
3 Inject 160 µL reconstituted ATP Reagent HS with dispenser 2 and measure light emission, I_{smp1}.
4 Measure light emission, I_{smp2} and immediately inject 10 µL 100 nmol/L ATP Standard, i.e. 1 pmol ATP, with dispenser 3 and measure light emission, $I_{smp+std}$ after mixing.
5 Calculate amount of ATP (pmol) in the sample by the equation:

$$ATP_{smp} = I_{smp1} / (I_{smp+std} - I_{smp2})$$

Each step should be performed for all samples in the plate before starting the next step. This is important to avoid too much cross-talk from the addition of ATP Standard. If only 2 dispensers are available Extractant B/S may be added manually.

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, but white microplates give higher signals. The cross-talk problem is in general overcome by reading the microplate immediately before and after the addition of the ATP Standard as described.
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 bacteria are suspended.
5 Always perform at least triplicate assays, since ATP contamination in cuvettes, microplates or on pipette tips may jeopardise the results.
6 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 HS should never exceed 1/4 (it may however be lower).
8 The volume of ATP Standard should always be 10 µL.
9 The higher the total assay volume the higher the sensitivity of the assay and the more accurate the results.
10 The light reading should be at least 10x higher after the addition of the ATP Standard as compared to before the addition. If not, the sample should be diluted.
11 If the cells in all samples are suspended in a similar medium and if this medium is not highly inhibitory to the luciferase reaction, it is possible to perform the assay without internal ATP standardisation of every assay, i. e. step 4 in the procedures described above can be omitted. Instead a single sample or the medium is assayed with and without ATP Standard and the ($I_{smp+std} - I_{smp}$) value is used for all samples.
12 Extractant B/S is available as a separate item, if the volume included in the kit is not adequate.

References

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

Liability

In case of problems the user is requested to contact the manufacturer with detailed description of the problems encountered. The claim will be analyzed and the outcome communicated to the claimer. The liability is restricted to the replacement of reagents consumed as a consequence of the kit not working according to specifications.

For laboratory use only. Not for drug, household or other use.