BioThema AB
Stationsvägen 17
S-136 40 Haninge
Sweden
VAT No. SE556303345401

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

ATP Hygiene Kit HS 266-221

Instructions for use to determine ATP in liquids

Intended use

The kit is intended for determination of ATP (adenosine triphosphate) on surfaces or in liquids. All living cells contain ATP. When cells die of natural causes, ATP is normally degraded. Most foodstuffs contain ATP at a level corresponding to the number of living cells in the foodstuff. Cleaning and disinfecting food processing equipment will leave small amounts of food residues containing ATP (partly within and partly outside cells). Such residues will provide an excellent growth medium for airborne or other microorganisms. Even if the equipment is sterile after cleaning and disinfecting, it may therefore contain large numbers of microorganisms within a few hours. Determination of ATP in e.g. the last rinsing water will tell how much food residue and the number of microorganisms were left after cleaning and disinfecting. It is possible to concentrate the sample by microfiltration to obtain a lower detection limit and to remove non-cellular ATP (cf. Procedural notes)

Assay principle

Before the assay, ATP must be released from the biological material. This is achieved with Extractant B/S, which extracts ATP from most cell types of animal as well as bacterial origin. Equal volumes of Extractant B/S and sample are mixed and an ATP reagent containing luciferase and luciferin is added. This results in the emission of light.

$$\text{ATP} + \text{luciferin} + O_2 \xrightarrow{\text{luciferase}} \text{AMP} + \text{pyrophosphate} + \text{oxyluciferin} + CO_2 + \text{light}$$

The intensity of the light is proportional to the amount of ATP and is measured in a luminometer. In a separate cuvette 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 pg (picogram i. e. 10^{-12} g).

Equipment

Any luminometer that takes cuvettes can be used. Cuvettes (outer diameter 12 mm) can be obtained from BioThema.

Kit contents

1. ATP Reagent HS (6 vials). Lyophilized reagent containing luciferase and luciferin. The level of luciferase in this reagent is so high that ATP is consumed at a rate of approx. 6%/min. This means that the light emission will decay with the same rate. It is therefore advisable to measure the light without any delay.
2. Diluent B (6 dropper bottles). Buffer used to reconstitute ATP Reagent HS.
3. Extractant B/S (2 dropper bottles). Extractant B/S has been shown to extract ATP from most cell types.
4. ATP Standard (2 dropper bottles). Contains ATP (10^{-8} mol/L, i. e. 507×10^{-8} g/L) dissolved in Extractant B/S.

The kit can be stored in the refrigerator (+4 ^{o}C) until the expiry date given on the labels. Reconstituted ATP Reagent HS should be stored at +4 ^{o}C and used within a week.

Reagent reconstitution

1. Remove screw cap and rubber stopper from ATP Reagent HS.
2. From the Diluent B bottle ensure that both screw caps (white and yellow) are removed at the same time by unscrewing the white cap.
3. Pour the contents of Diluent B dropper bottle into the ATP Reagent HS vial and then back again into the dropper bottle. Screw both caps (white and yellow) back on the dropper bottle.
4. Unscrew the yellow cap. The ATP Reagent HS is now ready to use.
5. Extractant B/S and ATP Standard are ready to use.

Assay procedure

The basic procedure is based on the use of dropper bottles. Care should be taken to hold the dropper bottle vertically. Ensure that the drops are produced at an even rate around one drop per second in one go. Since the reagents are delivered in dropper bottles, this is a convenient and surprisingly accurate procedure (cf. Procedural notes). It is essential to use similar volumes of sample and ATP Standard. It is also possible to perform the assay using normal pipettes but these must be ATP free.

Reagent blank (I_{blank1})

1. Add 3 drops (approx. 100 µL) of Extractant B/S to an empty cuvette.
2. Add 6 drops (approx. 400 µL) of ATP Reagent HS to the cuvette.
3. Measure the light signal from the cuvette corresponding to reagent blank, I_{blank1}.

Calibration of assays

1. Add 6 drops of ATP Reagent HS to a cuvette and measure the blank, I_{blank2}.
2. Add 3 drops of ATP Standard to the cuvette. Shake carefully and measure the light signal I_{stand}.

Assay of liquid samples

1. Add 3 drops (approx. 100 µL) of sample to 3 drops (approx. 100 µL) of Extractant B/S.
2. Add 6 drops (approx. 400 µL) of ATP Reagent HS.
3. Measure the light signal, I_{sample}.

Calculations

The ATP Standard contains 10^{-8} mol/L corresponding to $507x10^{-8}$ g/L. The added amount of ATP in 100 µL is therefore: 0.0001 L x 507 x 10^{-8} g/L = 507 pg. If the ATP Standard and sample is added in equal volumes (3 drops or approx. 100 µL) the ratio, k, between RLU and pg ATP can be calculated as:

$k=(I_{stand} - I_{blank2})/507$

By dividing measured light signals expressed in RLU with k (RLU/pg) one gets the amount of ATP expressed in pg.

Reagent blank (pg ATP) = I_{blank1} /k

Liquid samples (pg ATP) = I_{sample} /k

Amount of ATP on surface (pg ATP)

= I_{swab} /k - I_{blank1} /k

Procedural notes

1. The calibration with an ATP standard compensates for variations between different reagents and luminometers. Furthermore it is a check that luminometer and reagents are performing to expectations. Reconstituted ATP reagents gradually lose their activity. Calibration with an ATP standard makes it possible to use reagents that have lost up to half of their activity and still get the same result in picograms. Results expressed in picograms of ATP are comparable regardless of reagents and luminometers and make possible the exchange of information between different sites on cut-off limits.
2. The luciferase reaction is inhibited by strong chemicals. If you suspect your sample contains such chemicals, please contact BioThema for further information.
3. The reagent blank decreases by one order of magnitude every 40 min. This is particularly useful if the reagent has been contaminated during reconstitution.
4. The Extractant B/S has been evaluated in more than 25 different types of cells and in many complex biological samples. ATP is completely released in all bacterial strains tested except mycobacteria. Mycobacterial ATP can, however, be extracted with Extractant B/S, if the extraction is performed at 80-100 ^{o}C. Please contact BioThema for further information.
5. Before the extraction samples may be concentrated by microfiltration as follows:
   a. Suck up e.g. 60 mL sample in a disposable syringe.
   b. Run sample through a disposable microfilter (0.2 µ; 25 mm). Care should be taken to ensure that no air bubbles enter the microfilter.
   c. Slowly, for at least 10 seconds, elute cellular and microbial ATP from microfilter with 2 mL Extractant B/S using a 2 mL disposable syringe. A too rapid elution will give poor ATP yield.
6. The accuracy of the dropper bottles is surprisingly good. When used with a steady hand and held in a vertical position to dispense 3 drops the reproducibility typically corresponds to a coefficient of variation around 3%. The accuracy with 6 drops of reagents is not important, since 6-10 drops give almost the same result although more reagent is obviously consumed. It is recommended to calibrate your ”dropping technique” using a balance, before using this in routine analysis. In particular one must find a device/ technique by which sample is added in equal volume as the ATP Standard drops. Even though excellent results can be obtained with dropper bottles, a higher accuracy will result from using ATP free pipette tips. Please contact BioThema for sources of ATP free pipette tips..