

Product Information

Adenosine 5'-triphosphate (ATP) bioluminescent somatic cell assay kit

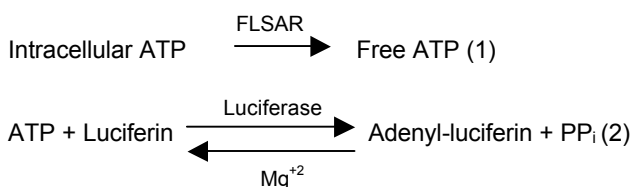
Catalog Number **FLASC**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

This kit may be employed for the bioluminescent determination of the adenosine 5'-triphosphate (ATP) released from a suspension of viable somatic cells. Estimates of cell concentrations may be calculated if it is assumed that the ATP content per viable cell remains fairly constant. The number of viable somatic cells is selectively counted because, as a cell dies, its ATP is rapidly degraded.¹

Generally, viable somatic cells will contain ~ 1 picogram (10^{-12} gram) or ~ 2 femtomoles (2×10^{-15} moles) of ATP per cell.²⁻⁶ A more exact ATP per cell ratio may be obtained for a particular cell line and growth medium from the literature, or by cell staining and counting the viable cells under a microscope before determining the ATP content. Viable somatic cell ATP may be determined as follows:



Reactions 1 and 3 are essentially irreversible and Reaction 2 is reversible with the equilibrium lying far to the right.^{7,8} When ATP is the limiting reagent, the light emitted is proportional to the ATP present, which is in turn proportional to the number of somatic cells in the sample.

Using the described procedure, this kit can measure the ATP released by fewer than 10 or as many as 2×10^5 viable somatic cells (sample containing from 400 to 8×10^6 cells per ml). This compares very favorably with the sensitivity obtained using a microscope with a hemocytometer, which may only detect down to $\sim 2 \times 10^5$ cells per ml. The results obtained with this kit will depend primarily on the amount of quenching by the medium in which the cells are suspended.

Components

Adenosine 5'-triphosphate (ATP) Assay Mix lyophilized powder containing luciferase, luciferin, MgSO_4 , DTT, EDTA, bovine serum albumin (BSA), and tricine buffer salts (Catalog Number FLAAM)	1 vial
Adenosine 5'-triphosphate (ATP) Assay Mix Dilution Buffer – lyophilized powder containing MgSO_4 , DTT, EDTA, BSA, and tricine buffer salts. (Catalog Number FLAAB)	1 vial
Adenosine 5'-triphosphate, disodium salt, hydrate (ATP Standard) – preweighed vial contains ~ 1 mg (2.0×10^{-6} mole). Actual ATP content is given on label. (Catalog Number FLAAS)	1 vial
Somatic Cell ATP Releasing Reagent 10 \times concentrated preparation, which increases membrane permeability to many small molecules. Cellular ATP is released almost immediately. (Catalog Number FLSAR)	1 vial

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use 0.2 μm filtered ultrapure water (17 $\text{M}\Omega\text{-cm}$ or equivalent) for preparation of reagents and throughout the procedure.

ATP Assay Mix Dilution Buffer – The contents of the Adenosine 5'-triphosphate (ATP) Assay Mix Dilution Buffer (Catalog Number FLAAB) vial should be dissolved in 50 ml of ultrapure water. This solution is stable for at least two weeks at 2–8 $^{\circ}\text{C}$.

ATP Assay Mix Stock Solution – The contents of the Adenosine 5'-triphosphate (ATP) Assay Mix vial (Catalog Number FLAAM) should be dissolved in 5 ml of ultrapure water to generate a stock solution with pH of 7.8. Mix by **gentle** inversion or swirling until dissolved. Allow the solution to stand in ice for at least one hour to assure complete dissolution. During this time a decrease in background may also be seen.

This solution is stable for at least two weeks when stored at 2–8 °C and protected from light. A slight decrease in light production and sensitivity may occur during this time. The stock solution may be dispensed in aliquots and frozen for future use. It is also stable for 2–3 freeze-thaw cycles.

ATP Assay Mix Working Solution – For most somatic cell ATP assays, dilution of the ATP Assay Mix Stock Solution ~25-fold with ATP Assay Mix Dilution Buffer is suggested. When cell samples are taken for assay directly from a cell culture, a 25-fold dilution of the ATP Assay Mix Stock Solution will measure the ATP released from 500 to 2×10^5 cells per assay, or 2×10^4 to 8×10^6 cells per ml. The light detected will not only depend on the number of cells and the amount of ATP per cell, but also on the degree to which the medium suspending the cells quenches the light produced.

For the most sensitive assays, the undiluted ATP Assay Mix Stock Solution can theoretically detect the ATP released from just one viable somatic cell. However, the lower limit of sensitivity is determined by the reliability of the cell sampling technique and by the amount of quenching and background observed due to the specific cell milieu.

ATP Standard Stock Solution – The ATP Standard Stock Solution is prepared by dissolving the contents of the Adenosine 5'-triphosphate, disodium salt, hydrate (ATP Standard) vial with 10 ml of ultrapure water. This stock solution is stable for at least 24 hours at 2–8 °C or over two weeks at –20 °C.

ATP Standard Working Solutions – Prepared by making serial dilutions of the ATP Standard Stock Solution with ultrapure water. The extent of dilution depends upon the assay sensitivity desired. The ATP Standard Working Solutions are stable for up to 8 hours when stored in ice.

1× Somatic Cell ATP Releasing Reagent – The Somatic Cell ATP Releasing Reagent (Catalog Number FLSAR) is a 10× concentrate and must be diluted 10-fold with ultrapure water before use. The 1× Somatic Cell ATP Releasing Reagent was found suitable for use for over 6 weeks when stored at 25 °C and is stable indefinitely at 2–8 °C. Freezing this solution did not affect its appearance or its ability to release ATP.

Sample Preparation – In preparing a sample for assay, it is important that the pH be adjusted to ~7.8. The use of 0.2 µm filtered ultrapure water is recommended if dilution of the sample solution is required or for dissolving a solid sample. It is advised not to use arsenate as the sample buffer since it tends to lower the sensitivity through quenching.⁹ Also, high salt concentrations in the sample will have a general inhibitory effect on the luciferase and will decrease sensitivity.^{10,11} The K_M for ATP increases with increasing ionic strength.

Storage/Stability

Adenosine 5'-triphosphate (ATP) Assay Mix (Catalog Number FLAAM) is stable indefinitely if stored desiccated at –20 °C and protected from light.

Adenosine 5'-triphosphate (ATP) Assay Mix Dilution Buffer (Catalog Number FLAAB) is stable indefinitely if stored desiccated at –20 °C.

Store the ATP Standard (Catalog Number FLAAS) desiccated at –20 °C.

Somatic Cell ATP Releasing Reagent (Catalog Number FLSAR) is stable indefinitely at –20 °C.

Procedure

To ensure reproducibility, low background, and maximum sensitivity, the following should be observed:

- Use 0.2 µm filtered ultrapure water (17 MΩ·cm or equivalent) for preparation of reagents and throughout the procedure.
- All assay vials, glassware, and pipette tips coming into contact with any of the samples or reagents should be as clean and as free from ATP and bacterial contamination as possible. An overnight soaking in 1 N HCl followed by a thorough rinse in ultrapure water is highly recommended. Allow to air dry free from drafts or heat in a drying oven.

- Pipette tips should never be used for more than one transfer and should not be allowed to come into contact with skin or any other contaminating surfaces before use.
- To prevent contamination of reagents and standards, do not return unused portions to their original containers.
- Contamination of standard diluent and highly diluted standards is the most common problem associated with ATP measurement. It cannot be overemphasized to take care in handling these solutions!
- Glass or plastic assay vials have been found satisfactory as reaction vessels, if precleaned as previously described. It has been reported that plastics such as polypropylene would be more suitable as an assay vial due to the absence of self-fluorescing impurities found in some glass, which may lead to a higher background. However, direct comparisons indicated little difference between the two types of vials.
- Generally, it will not be necessary to run a blank. But, when needed, a suitable blank may be prepared as described in the Appendix.

A series of up to 15 assays may be set up at one time.

1. Add 0.1 ml of ATP Assay Mix Working Solution to a reaction vial. Swirl and allow to stand at room temperature for ~3 minutes. During this period any endogenous ATP will be hydrolyzed, thereby decreasing the background.
2. To a separate vial containing 0.1 ml of 1× Somatic Cell ATP Releasing Reagent, add 0.05 ml of ultrapure water or whatever solution is used as the diluent for the ATP standards. Then add 0.05 ml of the cell sample to be assayed, swirl briskly, transfer 0.1 ml to the reaction vial, and immediately measure the amount of light emitted [$L_{(SAM)}$] with a luminometer.
Note: Relative to a simple ATP Standard assay, the addition of cell medium and 1× Somatic Cell ATP Releasing Reagent may cause some quenching. Although this quenching should not lower the effectiveness of the assay, it may be possible to decrease the amount of quenching by diluting the cell sample in sterile water or a dilute buffer having a pH of about 7.8.

3. The amount of ATP released may best be determined by running an internal standard. Add 0.1 ml of ATP Assay Mix Working Solution to a reaction vial and allow to stand at room temperature for ~3 minutes. To a separate vial containing 0.1 ml of 1× Somatic Cell ATP Releasing Reagent, add 0.05 ml of an appropriate ATP Standard and 0.05 ml of cell sample. Swirl briskly, transfer 0.1 ml to the reaction vial, and immediately measure the amount of light emitted [$L_{(SAM+IS)}$] with a luminometer.
Note: For best results, the amount of ATP in the added standard should contain about the same amount of ATP as that measured in the cell sample. In other words, the light emitted by the sample plus internal standard should be about twice that of the sample alone.

Results

Calculations

The amount of ATP in the cell sample may be calculated by the following equation:

$$ATP_{(SAM)} = \frac{ATP_{(IS)} \times L_{(SAM)}}{L_{(SAM+IS)} - L_{(SAM)}}$$

$ATP_{(SAM)}$ is the ATP in the cell sample (in moles)

$ATP_{(IS)}$ is the ATP in the added internal standard (in moles)

$L_{(SAM)}$ is the light emitted by the cell sample

$L_{(SAM+IS)}$ is the light emitted by the cell sample plus the internal standard.

If the amount of ATP per cell is known (obtained experimentally or from the literature), the number of viable cells per ml of original sample may be estimated by the following equation:

$$\text{Number of viable cells per ml} = \frac{ATP_{(SAM)} \times 40}{ATP \text{ per cell}}$$

References

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Appendix

Generally, it will not be necessary to run a blank. But, when needed, a suitable blank may be prepared as follows:

Add 0.1 ml of ATP Assay Mix Working Solution to a reaction vial and allow to stand at room temperature for ~3 minutes. To a separate vial, add 0.1 ml of 1× Somatic Cell ATP Releasing Reagent, 0.05 ml of ultrapure water (or ATP Standard diluent), and 0.05 ml of cell sample diluent (medium). Swirl briskly, transfer 0.1 ml to the reaction vial, and immediately measure the amount of light emitted [$L_{(B)}$].

The calculation should then be altered as follows:

$$ATP_{(SAM)} = \frac{ATP_{(IS)} \times [L_{(SAM)} - L_{(B)}]}{[L_{(SAM+IS)} - L_{(B)}] - [L_{(SAM)} - L_{(B)}]} \quad \text{or}$$

$$ATP_{(SAM)} = \frac{ATP_{(IS)} \times [L_{(SAM)} - L_{(B)}]}{L_{(SAM+IS)} - L_{(SAM)}}$$

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