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New Developments in Bioluminescence Technology: Detection of Microbial ATP in Biopharmaceutical In-Process Materials

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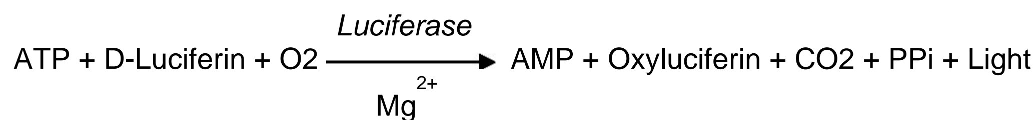
Abstract

ATP bioluminescence is a widely accepted method for rapid microbial screening of raw materials, in-process and finished products. Many bioprocessing samples contain high levels of non-microbial ATP that need to be reduced before microbial ATP can be reliably detected. Due to this limitation, standard ATP bioluminescence technology has not been widely applied to biopharmaceutical sample types. New developments address the need of the biopharmaceutical industry for a sensitive and reliable assay to proactively screen and manage raw and in-process materials for contamination. The biologics-specific assay uses traditional ATP bioluminescence in combination with a proprietary ATP-depleting reagent that effectively eliminates free and somatic ATP commonly found in biologic based sample types and allows for subsequent detection of microbial ATP as either a direct test or a 24h enrichment assay. The Celsis LuminASE™ Biologics reagent has proven to be effective on a series of samples typically found in the biopharmaceutical processing environment including media, cell culture and intermediates from vaccine production. Our data demonstrates that a short Celsis LuminASE treatment reduces sample background readings as high as 1,000,000 Relative Light Units (RLUs) to <1,000 RLUs and further reduced to <100 RLUs with an additional sample processing step. Rapid detection of microbial ATP in biopharmaceutical samples that contain non-microbial ATP allows for the early monitoring, analysis and control of raw and in-process materials and processing consistent with the philosophy of the process analytical technology initiative.

Introduction

Celsis technology utilizes the efficient light-producing enzyme system of ATP bioluminescence for the rapid detection of microorganisms. All actively respiring organisms contain adenosine triphosphate (ATP), which is used as the universal currency of free energy in biological systems. The luciferase enzyme hydrolyses ATP to AMP, releasing the stored energy as visible light. Luciferase can therefore be used to quickly detect the presence or absence of viable microorganisms in a sample by examining it for their ATP.

The mechanism of the light-producing reaction is:



The reaction is very efficient; every molecule of ATP can cause emission of a photon of green light. This provides a more rapid microbial detection system than waiting for visible colonies to grow on agar plates.

Some raw materials and end products may contain high levels of non-microbial ATP that can interfere with the bioluminescence reaction. This non-microbial ATP needs to be removed by use of an apyrase pretreatment. Celsis has developed a proprietary ATP-depleting reagent (Celsis LuminASE) that is capable of isolating and removing both free and somatic ATP, enabling the detection of microbial ATP in contaminated biopharmaceutical samples.

Objectives

Determine that ATP bioluminescence using the Celsis LuminASE (ATP-depleting enzyme) can:

1. Effectively remove both free and somatic ATP from two in-process pharmaceuticals and
2. Detect microbial contamination in these high level non-microbial ATP samples

Experimental Design

Samples
1. Concentrated (10x) Reformulated Viral Preparation
2. CHO cells – 1.0x106 per ml with 94% viability

Sample Preparation
The two products were prepared by adding 1 and 10 ml volumes to 99 and 90 ml volumes of Letheen neutralizing broth, respectively

Species Inoculated (Quanti-cult Plus®)

Microorganism	Average CFU
Staphylococcus aureus	24
Escherichia coli	16
Bacillus subtilis	2

Sample Baseline and Microbial Detection Study
1. Sample Baseline Study: Uninoculated product suspensions were prepared and assayed using only Celsis LuminASE buffer (no enzyme) and Celsis LuminASE to determine the ATP background of each of the products

2. Microbial Detection Study: Each product suspension was inoculated with the microbial species above, along with a sterile Letheen control. All samples were incubated at 30 ± 2°C for 24 hours, shaking at 200 rpm on an orbital shaker

Appropriate instrument and experimental controls were included:
Instrument controls: Instrument and Reagent Blanks and ATP Positive Control (set-up at time of assay)
Experimental controls: Broth control, product control (no bacteria), and microorganism control (no product)

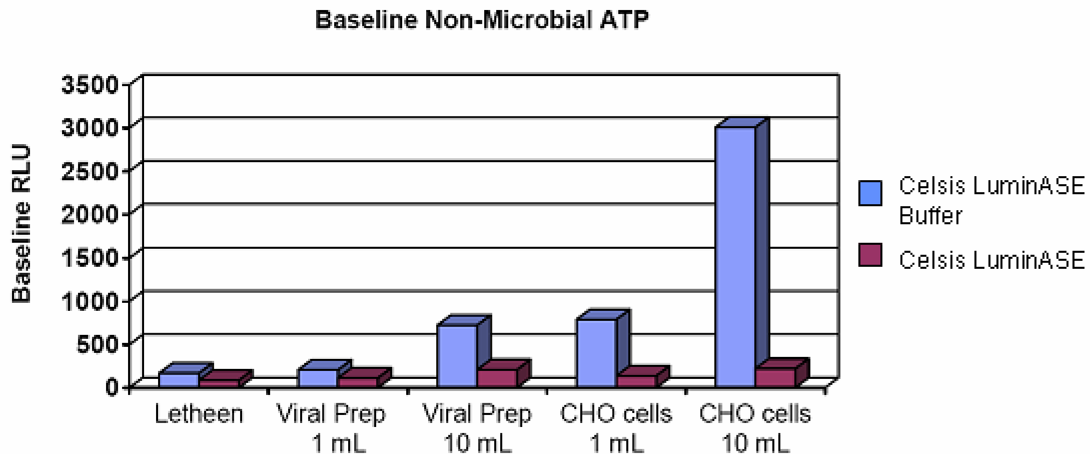
Instrumentation
Samples were assayed using a Celsis Advance™ luminometer

Reagents
RapiScreen™ Biologics 200 Kit , containing the following reagents:
Celsis LuminASE™
Celsis LuminASE™ Buffer
Celsis LuminEX™
Celsis LuminATE™
Celsis LuminATE™ Buffer

Sample Assay
50 µl of each incubated sample was pipetted into duplicate cuvettes
Celsis Advance.im automatically controls all injections and measurements
Results are presented as Relative Light Units (RLUs)

Data

Sample Baselines: Celsis LuminASE Buffer (no enzyme) versus Celsis LuminASE



The RapiScreen Biologics kit (Celsis LuminASE) can successfully decrease non-microbial ATP to Letheen broth baseline levels in both of the in-process products thus eliminating false-positive results.

Spiking Study (24h incubation):

Sample	RLU 1	RLU 2	Ave RLU	Celsis Result	Confirmation Plate
S.aureus Control	4005908	3945280	3975594	Positive	Positive
Conc. Viral Prep (1mL)	5400134	5298927	5349531	Positive	Positive
Conc. Viral Prep (1mL)	5199316	5362661	5280989	Positive	Positive
CHO cells (1mL)	5067219	5103850	5085535	Positive	Positive
CHO cells (10mL)	137	145	141	Negative	No Growth

Sample	RLU 1	RLU 2	Ave RLU	Celsis Result	Confirmation Plate
E. coli Control	1303372	1366699	1335036	Positive	Growth
Conc. Viral Prep (1mL)	1438175	1410144	1424160	Positive	Growth
Conc. Viral Prep(10mL)	2632660	2473947	2553304	Positive	Growth
CHO cells (1mL)	1451287	1377204	1414246	Positive	Growth
CHO cells (10mL)	1054936	999635	1027286	Positive	Growth

Sample	RLU 1	RLU 2	Ave RLU	Celsis Result	Confirmation Plate
B.subtilis Control	1342	1105	1224	Positive	Positive
Conc. Viral Prep (1mL)	1041	3002	2022	Positive	Positive
Conc. Viral Prep.(10mL)	1442	1473	1458	Positive	Positive
CHO cells (1mL)	110051	99983	105017	Positive	Positive
CHO cells (10mL)	152	166	159	Negative	No Growth

RapiScreen Biologics can successfully detect all 3 microorganisms in both in-process products using an enrichment of 1 ml product into 99 ml Letheen broth, and in the Concentrated Viral Prep sample using an enrichment of 10 ml product into 90 ml Letheen broth. The 10 ml CHO cell enriched sample could successfully detect *E. coli* but was inhibitory for growth of *S. aureus* and *B. subtilis* at this concentration.

Summary

- The Celsis LuminASE reagent was able to deplete non-microbial ATP in both products
- Depleting non-microbial ATP eliminates false-positive results and allows detection of low levels of microbial contamination in biopharmaceutical in-process samples
- The baseline study showed that the products did not interfere with the bioluminescence reaction
- The microbial detection study detected low level inoculation after 24h enrichment for all of the tested microorganisms in both products except for *S. aureus* and *B. subtilis* in the 10 ml CHO cell product sample. Lack of growth in this sample is most likely due to inadequate product neutralization that can be overcome by using a stronger neutralizing broth or increasing the broth volume

References

1. Mehta, H. Jeong, H., “Strategies for rapid microbial detection in the pharma/biotech industry.” *American Pharmaceutical Review* 6.1 (2003): 73-77.

2. Ashtekar, D., “Development of method, method optimization, and validation strategies for ATP based rapid method for determining the in-process bioburden.” 7th Annual advancing technologies in rapid methods conference (2003).