
Enhanced ATP Bioluminescence Using Adenylate Kinase Technology Versus Standard ATP Bioluminescence: A Comparative Study

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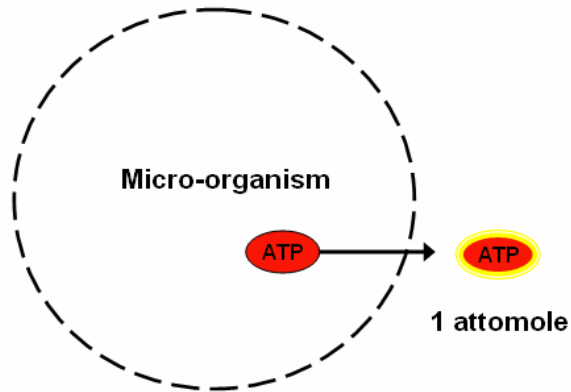
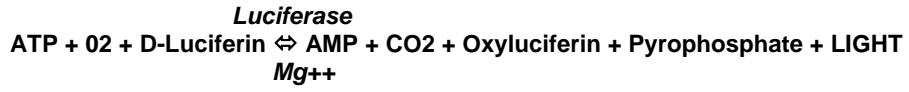
Abstract

ATP bioluminescence is a widely accepted method for the rapid screening of products, allowing the detection of microbial contamination within 24-48 hours. Conventional ATP bioluminescence detects the ATP of contaminating microbes; however the supplies of ATP are limited to the number of microbes present. Celsis has increased the sensitivity of conventional bioluminescence by using microbial adenylate kinase (AK) to increase the amount of ATP in a contaminated sample that ultimately reduces the time-to-result to 18 – 24 hours. After releasing AK from microbial cells, the enzyme converts purified ADP (supplied with assay) to ATP resulting in significantly higher quantities of ATP than that originally extracted from the microbial cells. In 25 minutes, AK can generate a three log increase in ATP levels over the levels originally contained in the cell significantly increasing assay sensitivity. A comparative study was performed on a variety of personal and homecare products. The products were artificially contaminated with a wide range of micro-organisms and tested using conventional and enhanced (AK accelerated) bioluminescence. The results demonstrated that enhanced assay results were achieved 25 – 50% faster than traditional bioluminescence. Additionally, in those instances where contamination was detected by both kits, the Signal (positive result) to Noise (background) ratio for AKuScreen™ was substantially higher, with 84% of samples tested using adenylate kinase technology having a S/N ratio in excess of 1000. Adenylate kinase technology offers a rapid assessment of product quality, facilitating a reduction in manufacturing lead times and the streamlining of the quality assurance process.

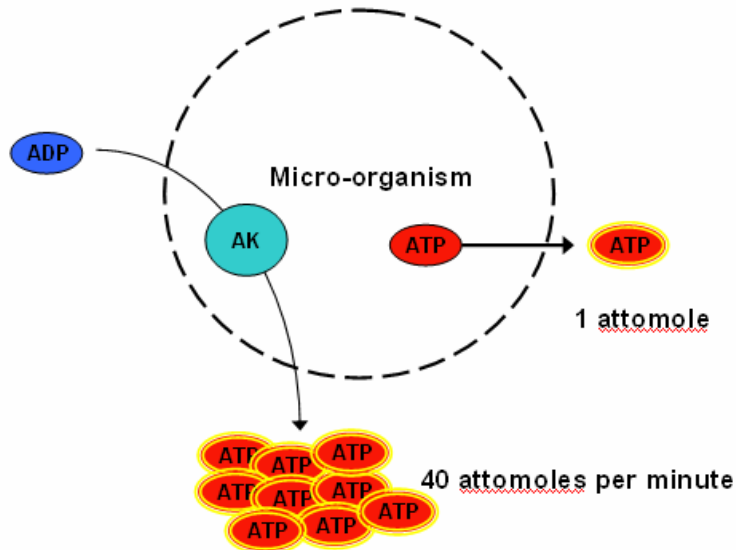
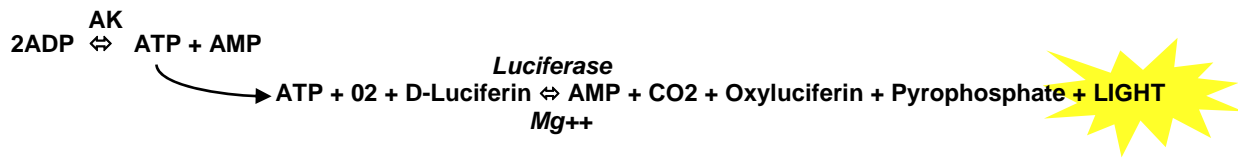
Celsis Rapid Detection Technology

- Based on widely accepted ATP bioluminescence technology
- A high-throughput, automated, walk-away system completes 164 samples in 1 hour
- Off-line incubation reduces instrument capacity requirement
- Flexible system and software
- Different samples and assays can be completed in a single workload
- Simple implementation
- Ongoing support provided to facilitate quicker implementation

Standard Bioluminescence



Enhanced Bioluminescence



Simple, Flexible Protocol

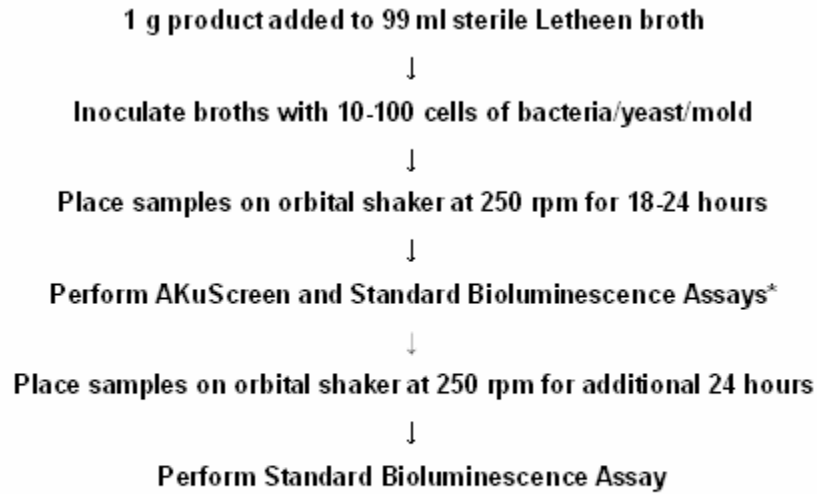


Non-filterable and filterable samples
 Non-destructive test (assay 50 μ l)
 Flexible sample size (0.1 to 10 g)

Celsis has developed a mold-specific protocol to allow 24 hour detection

	Standard Protocol	Mold Protocol
Prepare	1 g sample into 99 ml sterile enrichment broth (typically TAT or Letheen)	1 g sample into 99 ml sterile enrichment broth (typically TAT or Letheen)
Incubate	Shake at 30°C for 18 hours	Shake at 30°C for 24 hours
Disruption	NA	Add 10 ml sterile beads and antifoam. Place on linear shaker and homogenize for 30-40 minutes
Pipette	Pipette 50 μ l into cuvette	Pipette 50 μ l into cuvette
Assay	Celsis Advance.im automatically controls all injections and measurements	Celsis Advance.im automatically controls all injections and measurements

Experimental Design



*Appropriate instrument, broth and product controls included

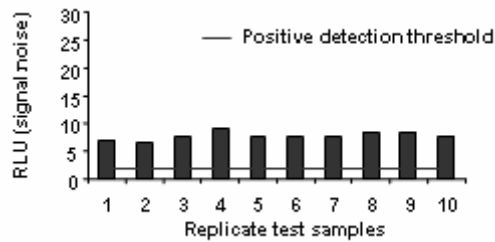
Data

AKuScreen detects bacteria and yeast in 18 hours

Microorganism	# Tested	Agreement (%)
<i>A. xylosoxidans</i>	4	100
<i>B. subtilis</i>	4	100
<i>B. cepacia</i>	4	100
<i>C. freundii</i>	4	100
<i>E. coli</i>	4	100
<i>E. cloacae</i>	4	100
<i>E. gergoviae</i>	4	100
<i>K. pneumoniae</i>	4	100
<i>M. luteus</i>	4	100
<i>P. aeruginosa</i>	4	100
<i>P. fluorescens</i>	4	100
<i>S. marcescens</i>	4	100
<i>S. aureus</i>	4	100
<i>S. epidemidis</i>	4	100
<i>S. haemolyticus</i>	4	100
<i>C. albicans</i>	4	100
<i>P. anomala</i>	4	100
<i>Z. baillii</i>	4	100

AKuScreen detects mold in 24 hours

Ten replicate cultures were inoculated with <50 *A. niger* spores each, incubated for 24 hours and tested with AKuScreen



In an internal Celsis study testing a wide variety of personal care products, high S/N ratios were obtained on a majority of products tested.

S/N	AKuScreen Assay	Standard ATP Assay
>1000	84	33
101-1000	6	21
10-100	6	38
<10	4	8

Summary

- Enhanced bioluminescence using the Celsis AKuScreen kit detects bacteria and yeast in 18 hours and mold in 24 hours.
- The Signal/Noise ratio is higher with AKuScreen than with standard bioluminescence assays which reduces uncertainty in results.
- Raw materials, in-process and finished goods can be released in 1 day with AKuScreen compared to standard bioluminescence (2 days).

References

1. Adenylate kinase technology is exclusively licensed to Celsis International plc by the UK Department of Defence and Scientific Testing Laboratories (DSTL) for use in the personal care, pharmaceutical and beverage industries.
2. Daane, L., Madden J., "Emerging Technology Assessment: Leveraging the power of adenylate kinase for rapid microbial testing," www.celsis.com, (2005).