charles river

MICROBIAL SOLUTIONS

Enzyme Technology:

How Amplifying the ATP Bioluminescence Reaction Produces Faster Time to Results

Key Points:

- An amplified ATP bioluminescence reaction achieves results 50% faster than standard ATP assay.
- Detection is possible in as little as 18 hours for bacteria and 24 hours for yeasts and molds.
- Reaction is not constrained by the finite amount of metabolic ATP available.
- Increased assay sensitivity achieves a stronger signal in less time.

Abstract

This case study compares the standard adenosine triphosphate (ATP) metabolite reaction with the amplified reaction of Celsis AMPiScreen®, which can reduce microbial hold times to 18-24 hours for microbial limits testing.

Situation

Leading pharmaceutical companies, as well as home and beauty product manufacturers around the world, are required to perform contamination testing to release their products safely to the market. In controlled manufacturing environments, these sterile and non-sterile products rarely generate positive results when screened for contamination, and thus rarely yield any counts to enumerate, so a simple absence/presence test is all that is needed. The gold standard of easy-to-use, reliable rapid microbial methods is ATP bioluminescence, a very sensitive technique already widely accepted and broadly used for quality control testing. However, companies are looking to find alternative methods that lead to increased time savings and better product compatibility, and have the ability to detect in the presence of non-microbial ATP for their raw materials, in-process and finished goods.

Solution

The Celsis AMPiScreen® assay is an amplified version of ATP bioluminescence that works up to 50% faster. ATP drives the reaction that produces light, which is, in turn, measured by a luminometer. ATP is present in all living cells as the transport molecule for most cell metabolic processes, including protein synthesis, replication, and motility. If microbial contamination is present, the reaction moves forward. Unlike traditional methods, which require 3-7 days of incubation to achieve visible growth, the standard ATP assay reduces time to detection to 24 hours for bacteria and 48 hours for fungi. Amplifying ATP gets results even faster.

Definitive detection is based on having a sufficient quantity of microbial ATP present to generate a clear, detectable light signal. The AMPiScreen® assay uses an enzyme-catalyzed reaction to overcome this limitation, thus reducing time to results for microbial contamination testing to as few as 18 hours for general detection of bacteria and 24 hours for yeast and mold. This is 25-50% faster than the already-rapid standard ATP method and 80% faster than traditional methods.

How It Works

The AMPiScreen® method of amplifying ATP is not constrained by the finite amount of metabolic ATP available in the standard bioluminescence assay. The generation or amplification of ATP beyond that which is inherent in the assayed sample is accomplished via a proprietary combination of enzymes, extractants and buffers, a luminometer and software to optimize the detection of microorganisms.

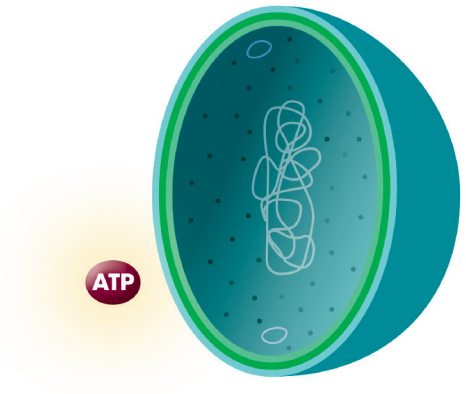
The amplification technology catalyzes and compounds the production of additional ATP. The total amount of ATP can then be more readily detected and measured using ATP bioluminescence. Given the ability of enzymes to effect reactions without being depleted or changed, it is possible to generate almost unlimited amounts of their products. Since ATP is one such product, and the reaction is essentially linear in nature, the longer the reaction is allowed to proceed, the more ATP is generated. This strengthens the bioluminescent signal and, therefore, the assay's sensitivity.

After 25 minutes, the amount of ATP can be 1,000 times more than was originally present, making the test enrichment period shorter and the overall time to result faster.

Results

Companies have found amplified ATP technology beneficial for shortening release times and verifying that their current good manufacturing processes and quality controls are working properly. The following examples show how companies that have benefited from adopting Celsis AMPiScreen® technology as part of their current quality operations.

ATP is present in all living cells, including microorganisms.



Celsis® uses enzymes to generate more ATP, quickly increasing the glow signal 1000-fold or more.

Raw Materials Testing

Not all companies will test their inbound bulk raw materials prior to use, mitigating their risk by relying on the quality of the supplier's practices. For one manufacturer of healthcare products, however, an investment in a Celsis® system enabled it to identify a potential problem and issue a precautionary and involuntary recall of certain lots of a preoperative product.

The company had been using its Celsis® system to release finished products faster and free up costly warehouse space. Once implemented in their quality control laboratory, the company was able to save so much time and identify additional areas where value could be added. One of these areas was testing raw materials, which quickly proved its worth, and it is now part of the company's testing protocol for all incoming materials.

A batch of chlorhexidine gluconate, purchased from an outside supplier, was found to contain *Burkholderia cepacia*[1], a Gram-negative organism known to pose a health risk to those with compromised immune systems. Since the ingredient was used in a pre-surgical skin preparation product, the ability to detect the problem within 24 hours enabled the company to quarantine the particular batch before it even made it to a patient's skin.

Products with Natural Ingredients

In recent years, the use of natural and organic ingredients has grown and, despite assumptions to the contrary, these products can be tested using the appropriate rapid method. To illustrate, we've summarized the experience of four different manufacturers of personal care products.

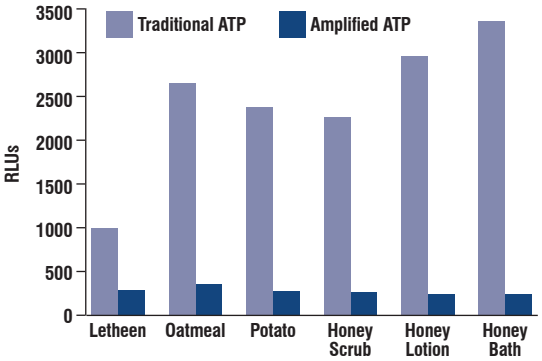
Products with natural ingredients may contain increased levels of non-microbial ATP, which must either be removed by use of an apyrase pre-treatment or masked by increasing the signal-to-noise ratio, the distance in relative light units (RLUs) between positive and negative results. The signal or positive result RLU divided by the noise or baseline RLU is a measurement of sensitivity. A higher signal-to-noise ratio reduces uncertainty created by user interpretation of results. The apyrase pre-treatment removes both free and somatic ATP, so it limits the ability to detect mold. In contrast, by amplifying the amount of microbial ATP in the sample, rapid methods using AMPiScreen® reagents can detect bacteria, yeast and mold within 24 hours, making it a better choice for these products.

Company A had a hand cream containing high concentrations of colloidal oatmeal; Company B's hand lotion had a high concentration of potato starch; Company C tested three products containing raw honey; and Company D had a moisturizing gel with high background ATP. The products were all reporting false positive results with the standard ATP assay.

Studies were performed on these manufacturers' products to determine the feasibility of decreasing the background noise as an option for masking non-microbial ATP. A baseline study was performed using both standard ATP and amplified ATP kits.

In the product study, all samples were prepared and tested following the companies' operating procedures for standard ATP bioluminescence, except that the samples were tested after only 24 hours of incubation. The assays were run using both standard ATP and AMPiScreen® reagents. AMPiScreen®, which optimizes the signal-to-noise ratio, significantly reduced the baseline for the product samples to low levels, thus enabling microbial contamination to be identified quickly and definitively.

Product Baselines



Conclusion

Whether comparing amplified ATP technology to standard bioluminescence or traditional methods, companies are finding the Celsis AMPiScreen® assay to be an effective screen for bacteria, yeast and mold, enabling them to release safe products to market faster.

References

1. Wheatley, M. "Supply chains at risk: Companies tap new technology to enact smart contingency plans – inbound and outbound." *Manufacturing Business Technology* (May/June 2009).