Product Data Sheet

Augmedium™

Catalog Numbers: 0123-0124

AthenaES®
a division of Athena Environmental Sciences, Inc.

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Product Description

Augmedium™ is a medium additive which conditions cells prior to induction of recombinant protein expression. This pre-induction conditioner increases the level of chaperone proteins which can improve the fraction of product that accumulates as soluble protein. Augmedium™ is specifically intended for use with strains in which the target protein accumulates as an insoluble aggregate. Augmedium™ is supplied as a powder for preparation of 50x concentrated stock solutions. It is available in 100ml and 500ml amounts.

Product Specifications

Unit Size	100mL Stock, 500mL Stock (50x solution)
Shipping	Ambient
Dry Powder Storage	Store at 0°C Stable for 2 years
Liquid Concentrate Storage Short-Term	Store at 4°C Stable for 2 months
Liquid Concentrate Storage Long-Term	Store at -20°C Stable for 6 - 12 months

Instructions for Use

Preparation of the 50x Stock:

1. Dissolve the contents of the 100mL container or 500mL container in 100mL or 500mL deionized water, respectively. Sterilize by filtration. Store at 4°C for short-term use and -20°C for long-term use.

Using Augmedium™ to Enhance Expression:

1. Inoculate 10mL of desired expression media (we recommend using Turbo Broth™ or Turbo Prime Broth™) supplemented with the appropriate antibiotic with a single colony of the expression strain. Incubate overnight at 37°C.

2. Use the overnight culture to inoculate six 250mL baffle bottom flasks containing 25mL medium each. Incubate at 30°C until the density reaches an OD_{600} of 0.9.

3. Add amounts of 0.5, 0.25, 0.125, 0.0625, and 0.03125mL of 50x Augmedium™ to five flasks, respectively. The sixth flask will be the untreated control. Incubate all flasks for 20 minutes.

4. To each of the 6 flasks add IPTG (or other inducer as per the expression system) to a final concentration of 1mM. Incubate for 3 hours.

5. Harvest the cultures by centrifugation at 3,000xg for 20 minutes. Store the pellets at -20°C or -80°C until ready to process.

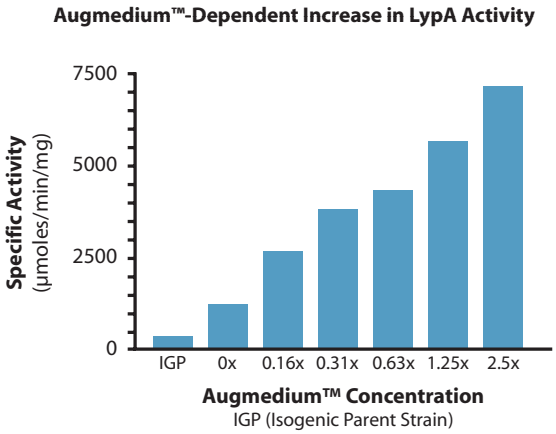

Figure 1. Medium-dependent accumulation of LypA after induction of expression. Augmedium™ was added at five different graduated concentrations. Cells were harvested after 3 hours of incubation. LypA activity was measured and the specific activity determined. A dose-dependent increase in enzyme activity with increasing Augmedium™ concentration is shown.

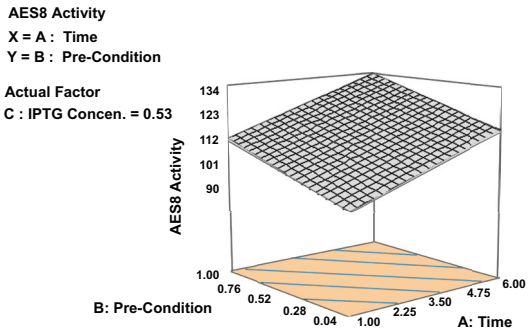

Figure 2. The above graph shows an increase in AES8 activity as a function of Augmedium™ concentration and induction time.

See full technical information at www.athenaes.com

Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.

6. Prepare cell-free extracts by mechanical, chemical or enzymatic disruption, depending on preference and requirements of expression system. Clarify the extract by centrifuging at 30,000xg for 30 minutes. Preserve the supernatant for next step.

7. Determine the amount of soluble protein in the supernatant by one of the following means:

   a: SDS-PAGE with Coomassie Blue or Silver Stain:

   Load equal amounts of protein in each lane. Compare the relative level of target protein accumulated.

   b: Immunoblot:

   Load equal protein per lane of a gel or well of a slot/dot blot. The primary antibody used can be to an affinity tag or to the target protein.

   c: Functional Assay:

   Perform a functional assay using equal amounts of protein in the assay.

8. Select the level of Augmedium™ which yields the highest level of target protein.

9. Repeat process with optimum conditions to express desired amount of protein.

*Note: It may be necessary to perform a time-course analysis to determine the optimum pre-condition period for any given protein and host/vector system.

References

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