MOK™

Media Optimization Kit™

The Media Optimization Kit™ determines the best medium formulation for maximizing accumulation of recombinant proteins expressed in *E. coli,* utilizing a series of Athena's superior proprietary expression media as well as two reference media.

AthenaES®

Athena Enzyme Systems™
888-892-8408
www.athenaes.com

Application Manual
V 3.0

MOK™

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AthenaES®

a division of Athena Environmental Sciences, Inc.

Athena Enzyme Systems™
1450 South Rolling Road
Baltimore, MD 21227
USA

T (MD): 410-455-6319
T (USA): 888-892-8408
F: 410-455-1155
aesinfo@athenaes.com

MOK™: Media Optimization Kit™
Application Manual

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Choosing the right culture conditions can be as important as choosing the right expression system to produce your protein.

Introduction

The Media Optimization Kit™ was designed to provide the researcher with a tool for determining the best available medium formulation for the production of recombinant proteins in *Escherichia coli*. The level of expression of a given protein is dependent upon the composition of the medium used. In the course of optimizing the production of recombinant proteins for clients, Athena's scientists have developed several media that were specifically formulated to maximize the accumulation of recombinant proteins expressed in *E. coli*. Four of these unique blends, Turbo Broth™, Superior Broth™, Power Broth,™ Hyper Broth™, are contained in the original Media Optimization Kit™ along with reference media LB Broth and Glucose M9Y. These blends of media have proven to be the most widely applicable formulations showing consistently superior performance over the traditional medium, LB Broth. With the increasing demand for non-animal derived materials, Athena's scientists developed *Animal Produce Free (APF)* formulations of the original Turbo, Superior, and Power Broths (Hyper Broth™ has always been *APF*.) The new version 3.0 MOK™ includes the original set of media as well as the *APF* media Turbo Prime Broth™, Turbo Prime-olate Broth™, Superior Prime Broth™, Superior Prime-olate Broth™, Power Prime Broth,™ and Power Prime-olate Broth™. Each of these media are *APF* Certified™.

Principle of the Kit

Historically, *E. coli* has been cultivated in LB Broth[1] and many gene expression protocols recommend this medium.[2] It should be pointed out that this medium was developed in the 1950's, nearly 20 years before the first gene was cloned and 30 years before recombinant protein expression became routine. Therefore, while LB Broth has proved to be very useful for cultivating E. coli, it was not specifically designed with the intention of maximizing the expression of recombinant proteins. Moreover, LB Broth is not normally supplemented with a carbon source nor is it buffered. Thus, the growth yields that one can obtain with LB are limited. Moreover, we have found that many proteins are not readily expressed in LB (Figure 1).

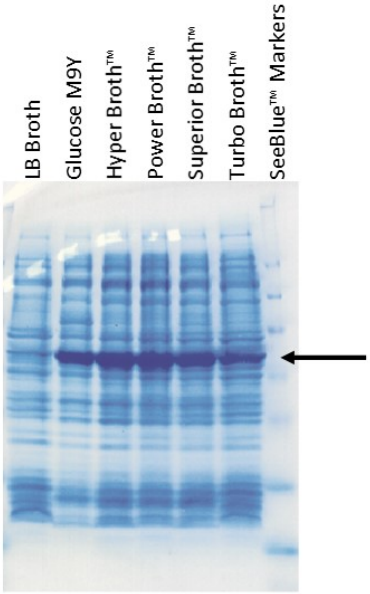

Figure 1. Media screen analysis by SDS-PAGE of a recombinant LypA esterase expressed in E. coli. The arrow denotes the recombinant protein. The protein was expressed in strain M15 grown in LB Broth, Glucose M9Y, Hyper Broth™, Power Broth™, Superior Broth™, and Turbo Broth™, lanes 1 to 6, respectively. Lane 7 is Novex SeeBlue™ marker proteins. For this particular protein, Hyper Broth™ was selected for production.

The principal purpose of the optimization kit is to allow for rapid identification of a suitable medium without the need for extensive optimization testing or development work. The test simply involves expressing the desired protein using each of the media provided in the kit. The relative level of expression of the target protein, after induction of expression, is determined by SDS-PAGE using a qualitative assessment. In some cases an immunoblot or functional assay may be appropriate.

The optimum conditions for the expression of a recombinant protein requires attention to four culture-related parameters. These include the strain employed, the medium composition, the time-course of induction, and the concentration of inducing agent. We recommend performing the culture optimization tests in the order listed. Thus, the Media Optimization Kit™ a should be used after selecting the best available strain.

Kit Components

Individually packaged, ready-to-use powdered media to make 1 liter of each broth.

PERK™ Kit Components					
Component	**Cat. Number**	**Component**	**Cat. Number**	**Component**	**Cat. Number**
LB Broth (Miller)	0103	Turbo *Prime* Broth™	0110	Power *Prime-olate* Broth™	0162
APF LB Broth (Miller)	0133	Superior *Prime* Broth™	0111	Glucose M9Y	0108
Turbo Broth™	0104	Power *Prime* Broth™	0112	Glucose Nutrient Mix	0109
Superior Broth™	0105	Turbo *Prime-olate* Broth™	0160		
Power Broth™	0106	Superior *Prime-olate* Broth™	0161		
Hyper Broth™	0107				
Reagents needed but not provided: Glycerol					

Protocols

Preparation of Liquid Media

1. Dissolve the contents of each of the media packets in deionized water as directed on the individual packets.
2. Add 4 mL of glycerol to all versions of the Turbo Broths and Power Broths.
3. Filter sterilize using a 0.2µm filter. Store at 4°C. Media may be autoclaved, however, precipitates may form in *APF* media which are innocuous.
4. Di spense desired volume into appropriate bottles or flasks. (We recommend 2 x 500mL glass bottles.)
5. Dissolve the contents of the Glucose Nutrient Mix in 100 mL deionized water and filter sterilize using a 0.2µm filter.
6. Add 50 mL of the sterile Glucose Nutrient Mix to 1 liter of Hyper Broth™ and 20 mL to 1 liter of Glucose M9Y using aseptic technique.
7. Add sterile antibiotics as needed.

Media Screening Protocol

1. Materials

1.1. 25 mL of each culture medium in 250 mL baffle bottomed flasks.

1.2. Wash Buffer: 40 mM sodium phosphate pH 7.5, 150 mM NaCl

1.3. 2x SDS-PAGE Loading Dye: 125 mM Tris-Cl pH 6.8, 4% SDS (w/v), 0.005% bromophenol blue (w/v), 20% glycerol (v/v), 5% ß-mercaptoethanol (v/v)

1.4.. Tris-Bis SDS-polyacrylamide gel of appropriate composition.

2. Methods

2.1. Inoculate a single colony of the recombinant strain into 10 mL of LB Broth in a shake flask with baffle bottom. Incubate at 37°C overnight.

2.2. Inoculate 25 mL of each of the six media with 1 mL of the overnight culture. Incubate the cultures at 37° C until the OD_{600} reaches 1.0.

2.3. Remove a 1 mL sample ("pre-induction"), harvest the cells in a pre-weighed microfuge tube, and store at -20°C.

2.4. Add inducer (see Tip 1) and continue incubating for 3 hours (see Tip 2).

2.5. Remove a 1 mL sample ("post-induction") and process as in step 2.3.

2.6. Harvest the remainder of the culture, wash with 10 mL of wash buffer, determine the mass of the cell pellet, and store the cell pellets at -80° C. (see Tip 3.)

2.7. Analyze for expression of the target protein as follows:

2.7.1. To determine protein production per mL of culture:

2.7.1.1. Suspend the cell pellets from the pre- and post-induction samples in 0.5 mL of water.

2.7.1.2. Mix 5 µL of each cell suspension with 7.5µL water and 12.5µL 2x SDS-PAGE loading buffer. Heat at 100° C for 5 minutes and load 10 µL per lane of acrylamide gel.

2.7.2. To determine the relative level of expression:

2.7.2.1. Suspend the cell pellets from the pre- and post-induction samples in water to a density of 10 OD/mL

2.7.2.2. Mix 5 µL of each cell suspension with 7.5µL water and 12.5µL 2x SDS-PAGE loading buffer. Heat at 100° C for 5 minutes and load 10 µL per lane of acrylamide gel.

2.7.3. Stain the gel with Coomassie Blue, colloidal Coomassie Blue or Silver stain. *(see Tips 4 and 5).*

3. Interpretation

3.1. After staining the gel, observe each lane and compare the "pre-induction" sample with the "post-induction" sample from each medium. Elevated expression is indicated by the presence of a unique polypeptide band corresponding to the molecular mass of the target protein in the "post-induction" sample. An example using the human protein MP87 is shown in Fig. 2.

3.2. Compare the level of target protein from cells grown in each of the six media. Select the medium which produces the highest level of target protein per mL of culture. Figure 1 shows the results of a media screening experiment.

3.3. If two or more media give the same level of production per mL, then use the analysis of 2.7.2 to select the medium with the highest relative level of expression.

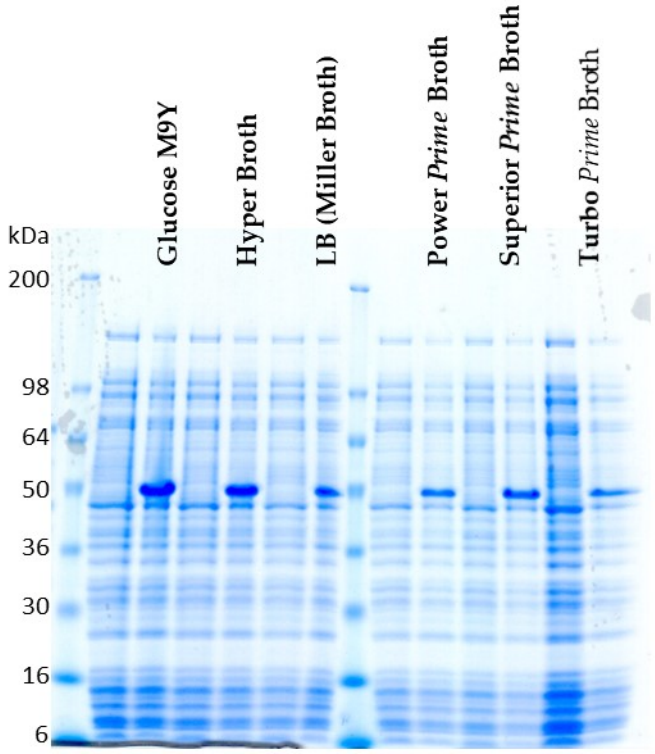

Figure 2. SDS-PAGE analysis of MP87 accumulated in strain HMS174(DE3). MP87 is a human protein with anti-inflammatory properties. The gel was a 4-20% Acrylamide Tris-glycine gradient gel resolved in Tris-glycine-SDS running buffer. The polypeptides were visualized using Gel Code Blue (Pierce Chemical). Lane 1 and 8—SeeBlue Markers; Lanes 2 and 3—Glucose M9Y (Cat. No. 0109), t0 and t3; Lanes 4 and 5—Hyper Broth™ (Cat. No. 0107), t0 and t3; Lanes 6 and 7—LB (Miller) Broth (Cat. No. 0103), t0 and t3; Lanes 9 and 10—Power Prime Broth™ (Cat. No. 0122), t0 and t3; Lanes 11 and 12—Superior Prime Broth™ (Cat. No. 0121), t0 and t3; Lanes 13 and 14—Turbo Prime Broth™ (Cat. No. 0120), t0 and t3.

Tips of the Trade

Tip 1: Inducer Optimization

The inducer used will depend on the expression system employed. The concentration of inducer is strain-dependent and the optimum concentration should be determined empirically. For *lac*P-based expression systems, 1 mM IPTG is a good starting point for the media optimization.

Tip 2: Inclusion Bodies

Some recombinant proteins are expressed in *E. coli* as insoluble particles known as inclusion bodies. The formation of inclusion bodies cannot be predicted, but are indicated by the presence of intracellular refractive objects when viewed under oil immersion microscopy.[3] The formation of inclusion bodies will not affect the results of the media screen, because the analysis is done on whole cell extracts prepared by boiling the cells in sodium dodecylsulfate. This procedure completely denatures inclusion bodies as well as membrane and cytoplasmic proteins. (In some instances lowering the temperature after induction can increase the amount of soluble protein recovered. This should be determined experimentally.)

Tip 3: Cell-free Extract

Once the medium yielding the highest level of expression has been determined, the cell paste can be used to prepare a small-scale cell-free extracts. Use sonication, lysozyme/freeze-thaw, bead mill, or microfluidizer. Evaluate soluble and insoluble fraction.

Tip 4: SDS-PAGE gels

Coomassie Blue stain should be sufficient to visualize the expression of a recombinant protein and staining is quantitative using image analysis software. Silver stain, while allowing detection of smaller amounts of protein, is more difficult to interpret and should only be used for examining whole cell extracts which are separated by SDS-polyacrylamide gels that are 20 cm in length or longer. The long gel will give bett er resolution of individual polypeptide bands.

Tip 5: Alternative Techniques

Alternative detection techniques should be applied to the media screen analysis when available. Immunoblot and functional assays should be employed as available particularly if the identity of the target is in question or if expression of a functional protein is critical. Care should be taken when using functional assays by first demonstrating that there is no interfering activity contributed by the host. In most cases, the SDS-PAGE analysis is a suitable method of choice during the early stages of developing the expression system. Immunoblots should be used when the Coomassie blue stain does not reveal any expressed protein.

Technical Assistance

The scientific staff of the Athena Enzyme Systems™ are specialists in the expression of recombinant proteins in microbial systems. They have extensive expertise in all aspects of protein expression from the construction of expression vectors to the commercial production of recombinant proteins. No matter what your question, please feel free to ask them for help. A technical support scientist can be reached at support@athenaes.com.

Product Use Limitations

The Media Optimization Kit was designed and is sold for research use only. It should not be used for human diagnosis or drug use or administered to humans unless expressly cleared for that purpose by the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials contained in the kit. Athena's dehydrated media products are manufactured in a GMP Compliant, ISO 9001:2015 certified facility.

Product Warranty

AthenaES™ guarantees the quality and performance of the media and reagents contained in this kit for the cultivation of *E. coli*. The suitability of a medium formulation or additive for a particular use is the responsibility of the end user. No guarantee is made that a given protein will be expressed when applying this kit. AthenaES™ will replace the product free of charge if it does not conform to the stated specifi cations. Notice for replacement must be received within 60 days of opening the product.

References

1. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

2. Luria, S. E., and J. W. Burrous. 1955. Hybridization between *Escherichia coli* and Shigella. J. Bacteriol. 74:461-476.

3. Broedel, Jr., S. E., S. M. Papciak, and W. R. Jones. 2001. The selection of optimum medium formulations for improved expression of recombinant proteins in *E. coli.* Athena Enzyme Systems Technical Bulletin , January 2001.

Ordering Information

To place an order:

Phone: 1-888-892-8408 (in U.S. and Canada), 1-410-455-6319 (International)
Fax: 410-455-1155
Email: orders@athenaes.com (preferred method)
Website: www.athenaes.com/orders-page.html
Or visit our website to order through one of our international and domestic distributors.

When placing an order, please provide the following:

- Institution name and customer service account
- Purchase order number
- Catalog number(s) or names of products and quantity of item(s)
- Billing and shipping address
- Contact name and telephone number

Delivery:

Telephone orders received Monday through Friday before 12 noon will be shipped that day. All other orders will be shipped the next business day, unless otherwise stipulated.

MOK™ Replacement Products Information

Catalog Number	Product	Size
0103	LB Broth (Miller)	500 g
0133	*APF* LB Broth (Miller)	500 g
0104	Turbo Broth™	500 g
0105	Superior Broth™	500 g
0106	Power Broth™	500 g
0107	Hyper Broth™	500 g
0108	Glucose M9Y	500 g
0109	Glucose Nutrient Mix	150 g
0110	Turbo *Prime* Broth™	500 g
0111	Superior *Prime* Broth™	500 g
0112	Power *Prime* Broth™	500 g
0160	Turbo *Prime-olate* Broth™	500 g
0161	Superior *Prime-olate* Broth™	500 g
0162	Power *Prime-olate* Broth™	500 g