PERK™

Protein Expression Rescue Kit™

The Protein Expression Rescue Kit™ maximizes the amount of soluble protein produced during expression of recombinant proteins in *E. coli*. This comprehensive kit includes samples of Athena's proprietary expression media and superior medium additives as well as two reference media.

AthenaES®

Athena Enzyme Systems™
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Application Manual
V 1.1

PERK™

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AthenaES®

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PERK™: Protein Expression Rescue 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 Protein Expression Rescue Kit™ was designed to provide the researcher with a tool for determining the best available culture conditions for the production of recombinant proteins in *Escherichia coli*. Experience has shown that the accumulation of a given protein in the cytoplasm is dependent upon several factors. These include the host strain, the composition of the medium and the incubation conditions used. In the course of optimizing the production of recombinant proteins for clients, Athena's scientists have developed an algorithm that was specifically designed to maximize the accumulation of recombinant proteins expressed in *E. coli*. Figure 1 depicts the experimental flow chart followed for this algorithm.

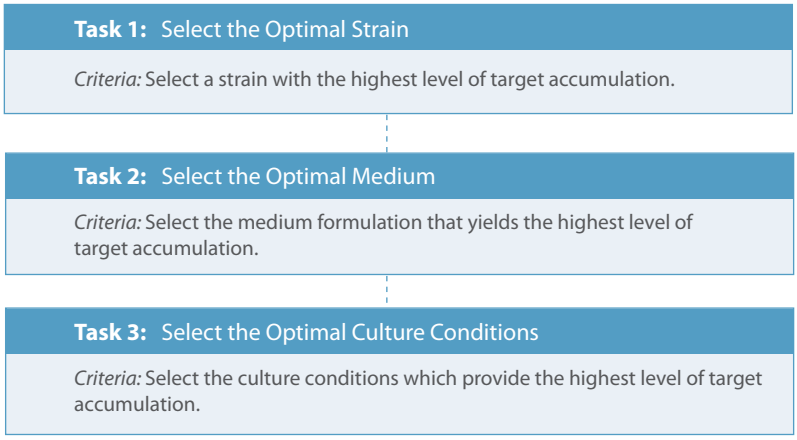

Figure 1: Optimizing Production of Recombinant Proteins

Principle of the Kit

Many lines of research have lead to the development of a plethora of host-vector systems for the over-expression of proteins in *E. coli.* A review of the literature will quickly reveal that it is not possible to accurately predict which host-vector system is best suited for a given protein. While systems that employ strong promoters are commonly the first choice, the resulting hyper-expression is often not effective for producing active soluble protein. One approach for improving expression is to apply alternative host strains. Thus, the first step of the PERK™ algorithm is to test alternative strains.

Historically, *E. coli* has been cultivated in LB Broth and many gene expression protocols recommend this medium.[1] 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.[2] Therefore, while LB Broth has proved 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. The growth yields that one can obtain with LB are limited and we have found that many proteins are not readily expressed in LB.[3] The second step of the PERK™ process is, therefore, to identify the medium formulation that gives the highest level of expression.

In the final step of the PERK™ optimization process, culture conditions leading to the maximal accumulation of the target protein are determined. This is an iterative process which involves first identifying the critical factors for expression and then optimizing the cultivation conditions around the critical factor(s). The factors that are considered by the PERK™ are incubation temperature during induction, inducer concentration, duration of induction, and the use of media additives. The PERK™ tests two additives which are designed to either increase the biomass production, using fructose as the carbon source, or to induce the expression of chaperone proteins by invoking a mild chemical and oxidative stress response.

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**
LB Broth (Miller)	0103	Glucose M9Y	0108
Turbo Broth™	0104	Glucose Nutrient Mix	0109
Superior Broth™	0105	LB*Booster (20mL)	0125
Power Broth™	0106	Augmedium™ (25mL)	0123
Hyper Broth™	0107		
Reagents needed but not provided: Glycerol			

Protocols

Preparation: Media

1. Dissolve the contents of each of the media packets in deionized water as directed on the individual packets.
2. Add 4mL of glycerol to the Turbo Broth™ and Power Broth™ solutions.
3. Dispense desired volume into appropriate bottles or flasks. (We recommend 2 x 500mL glass bottles.)
4. Autoclave at 121°C for 20 min. The autoclaved media without antibiotics are stable for 6 months at 4°C.
5. Dissolve the contents of the Glucose Nutrient Mix in 100mL deionized water and filter sterilize using a 0.2µm filter.
6. Add 50mL of the sterile Glucose Nutrient Mix to 1 liter of Hyper Broth™ and 20mL to 1 liter of Glucose M9Y using aseptic technique.
7. Add sterile antibiotics as needed.

Preparation: Reagents

1. Dissolve the contents of the LB*Booster™ packet in 20mL deionized water and filter sterilize using a 0.2µm filter. The stock solution will be 20x. Store at 4°C.
2. Dissolve the contents of the Augmedium™ container in 25mL deionized water and filter sterilize using a 0.2µm filter. Aliquot 5mL portions into sterile 15mL conical tubes and store at -20°C. The stock will be a 50x solution. For use, snap thaw at 37°C and store at 4°C for no more than five (5) days.

Task 1: Strain Selection Protocol

1. Materials

1.1. Three different strains to be tested. See Table 1 for suggested strains.

1.2. Turbo Broth™

1.3. Extraction Buffer: 50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole, 1 mM PMSF (or 0.1 mM AEBSF)

1.4. Lysozyme and DNaseI (or equivalent substitute)

1.5. 5x SDS-PAGE loading dye with β-mercaptoethanol

Selected Strains	
Strain	**Genotype**
HMS174	*F⁻ recA, hsdR*(r_{K12}^-,m_{K12}^+) (Rif^R)
BLR	*F⁻ ompT, hsdS*(r_B^-,m_B^-), *gal, dcm Δ(srl-recA)*306::Tn10 (Tet^R)
AG1	*F⁻ recA1, endA1, gyrA96, thi, hsdR17, glnV, relA1*
SURE	*hsdR, mcrA, mcrB, mrr, endA1, glnV, thi, gyrA96, relA1, lac, recB, recJ, sbcC, umuC, uvrC/F' traD36, proAB lacI^q Δ(lacZ)*M15, Tn10 (Tet^R)
JM109	*Δ(lac-proAB), recA1, endA1, gyrA96, thi, hsdR17, glnV/ F' traD36, proAB lacI^q Δ(lacZ)*M15
HB101	*hsdR, hsdM, glnV, ara14, galK2, lacY1, proA2, rspL20, xyl-5, mtl-1, recA13, mcrB*

Table 1. Selected *E. coli* strains useful for the expression of recombinant proteins.[1]

1. There are many other strains that are suitable for expressing heterologous proteins in *E. coli.* This list represents those strains which the R&D team at AthenaES™ has had the most consistent success with for producing recombinant proteins.

2. Methods

2.1. Introduce the expression vector into the three strains.

2.1.1. If competent cells can be purchased or are readily available, follow the manufacturer's protocol for transformation.

2.1.2. If competent cells are not available, use Supplemental Protocol No. 1 to introduce the plasmids into the respective strains.

2.2. Grow the cells to induce expression of the target protein.

2.2.1. Inoculate 3- 2mL of Turbo Broth supplemented with the appropriate antibiotic with a single colony of each strain.

2.2.2. Grow cultures overnight, shaking, at 37°C.

2.2.3. Inoculate 3- 25mL Turbo Broth in 250mL baffle bottomed flasks with each overnight culture, record the absorbance at 600nm and incubate at 37°C, with shaking at 250rpm.

2.2.4. At 0.5 or 1 hour intervals, record the absorbance at 600nm.

2.2.5. When the culture density reaches 1.0 ± 0.2, remove a 10mL sample from each culture flask, harvest the cells in pre-weighed tubes, record the cell mass and freeze the cell pellets at -80°C.

2.2.6. Add IPTG to 1 mM (or other inducer depending on the expression vector) and continue incubating for 3 h. *(See Tip 1.)*

2.2.7. At 3 h post-induction, remove 10mL samples and process the cells as in step 2.2.5.

2.2.8. Dispose of the remainder of the culture according to the requisite biosafety regulations for your institution or harvest the cells and store at -80°C.

2.3. Prepare whole cell, soluble and insoluble extracts.

2.3.1. Suspend the cell pellets in 2 ml/g cells of Extraction Buffer.

2.3.2. Add lysozyme and DNase I to 1.0 and 0.1 mg/ml, respectively. Mix well.

2.3.3. Incubate the cell mixture on ice for 30 min with stirring.

2.3.4. Prepare a whole cell extract by mixing 5µl treated cells with 35µl water and 10µl 5x SDS-PAGE loading dye. Heat at 100°C for 5 min and reserve. *(See Tip 2.)*

2.3.5. Lyse the cells with three cycles of freezing (on a dry ice-ethanol bath) and thawing (at 37°C).

2.3.6. Clarify the extracts by centrifuging at 4°C at 27,000 xg for 30 min. Decant supernatants to clean containers. These are the soluble extracts.

2.3.7. Dissolve the pellets in (2 ml/g pellets) of 8 M urea, 100 mM Na2HPO4, 10 mM Tris-Cl, 10 mM DTT pH 8.0. Store at room temperature. These are the insoluble extracts.

2.3.8. Store the supernatants at -80°C.

2.4. Analyze the extracts by SDS-PAGE. If a functional or immunoblot assay is available, these should be used to complement the stained gel result.

2.4.1. Determine the protein concentration of the soluble and insoluble fractions using the Bradford or BCA assay.

2.4.2. Prepare the soluble and insoluble samples for SDS-PAGE by mixing an aliquot of the respective sample with water and 5x SDS-PAGE loading dye to give a final protein concentration of 2 mg/ml. Heat the soluble fraction samples at 100°C for 5 min. Do not heat the insoluble samples as they contain urea.

2.4.2.1. There should be a total of 18 samples: 3 whole cell, 3 soluble and 3 insoluble fractions taken at t0 and t3 post-induction.

2.4.3. Load 10µl of each sample on a 4-20% acrylamide gradient Tris-Glycine gel (or other gel buffer system) such that the t0 and t3 samples for each corresponding fraction are next to each other.

2.4.4. Electrophorese and stain the gel with Coomassie Blue, colloidal Coomassie Blue or silver stain. *(See Tips 4 and 5.)*

3. Results Interpretation

3.1. In the lanes corresponding to the whole cell extracts, identify the strain with the highest degree of differential expression. 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" samples. This is the strain that produced the most target protein.

3.2. In the lanes corresponding to the soluble and insoluble fractions, identify the strain which produced the most target protein.

3.3. Select the strain that either produced the most protein or the strain that produced the most soluble protein depending on the intended use of the protein and whether the recovery of inclusion bodies presents a potential problem.

Task 2: Media Screening Protocol

1. Materials

1.1. Strain selected from Step 1.

1.2. 25mL of each of the six culture media provided in 250mL baffle bottomed flasks.

2. Methods

2.1. Inoculate a single colony of the selected recombinant strain into 25mL of LB Broth and incubate at 37°C overnight.

2.2. Inoculate 25mL of each of the six media with 2mL of the overnight culture. Incubate the cultures at 37°C until the OD_{600} reaches 0.8 ± 0.2.

2.3. Remove a 1mL sample ("pre-induction") from each of the six flasks, harvest the cells in a microfuge tube, and process as in step 2.7.

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

2.5. Remove 1mL samples ("post-induction"), record the OD_{600}, harvest the cells in microfuge tubes, and process as in step 2.7.

2.6. Harvest the remainder of the culture, 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. Resuspend the cell pellets from the pre- and post- induction samples in 500µL water.

2.7.1.2. Mix 5µL of each cell suspension with 15µL water and 5µL 5x SDS-PAGE loading buffer. Heat at 100°C for 5 min. and load 10µL per lane.

2.7.2. To determine the relative level of expression:

2.7.2.1. Resuspend the cell pellets from the pre- and post-induction samples in water to a density of 10 OD_{600}/ml.

2.7.2.2. Mix 5µL of each cell suspension with 35µL water and 10µL 5x SDS-PAGE loading buffer. Heat at 100°C for 5 min. and load 10µL per lane.

2.7.3. To determine the relative level of soluble protein production:

2.7.3.1. Prepare extracts as in Step 1: Strain Selection Protocol, step 2.3 or by sonication.

2.7.3.2. Load 5µg of total protein per lane of a 10cm gel (4-20% Tris-Glycine gradients are recommended).

2.7.4. Stain the gel with Coomassie Blue, colloidal Coomassie Blue or silver stain. *(See Tips 4 and 5.)*

2.7.5. Use functional and immunoblot assays to complement the SDS-PAGE analysis and to verify the identity of the protein.

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.

3.2. Compare the level of target protein obtained from cells grown in each of the six media. *Select the medium which produces the highest level of target protein per ml of culture.*

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 which gives the highest relative level of expression.

3.4. Compare the level of target protein obtained in the soluble fractions for each of the six media. *Select the medium which produces the highest level of target protein in the soluble fraction. (See Tip 2.)*

Task 3: Determine the Critical Factors for Expression

1. Materials

1.1. Expression strain

1.2. Medium – identified in Step 2

1.3. 20x LB*Booster™

1.4. 50x Augmedium™

1.5. Two shaking incubators set at 37°C and 27°C.

1.6. 1M IPTG

2. Methods

2.1. Inoculate a 50 mL culture with a single colony of the expression strain and incubate at 37°C overnight.

2.2. Inoculate 500 mL of fresh medium with the overnight culture.

2.3. Incubate at 37°C until the absorbance at 600 nm reaches 1.0 ± 0.2.

2.4. Remove a 1mL sample, harvest the cells by microfuging and suspend the cells in water to give 10 OD/ml. Store at -20°C.

2.5. Dispense 25mL of the culture into each of 16 baffle bottomed flasks (250mL).

2.5.1. Alternative Protocol 1: This experiment can be done in sets of eight cultures. Reduce the culture volume to 250mL.

2.5.2. Alternative Protocol 2: If an incubator that can shake microtiter plates is available, this experiment can be done using 24-well or 6-well plates. Adjust the volume of medium accordingly.

2.6. Add the requisite volume of 50x Augmedium™ to give the prescribed final amount according to Table 2.

2.7. Transfer the cultures to the indicated temperatures and incubate for 20 min.

2.8. Add IPTG and LB*Booster™ to the final concentrations shown in Table 2.

2.9. Incubate for 3 hours at the temperatures indicated.

2.10. Remove 1mL samples and process as in step 2.4.

Critical Factors that Affect Accumulation of a Target Protein				
Culture No.	IPTG (mM)	Temp. (˚C)	LB*Booster™ (x)	Augmedium™ (x)
1	1	37	1	0.1
2	1	27	1	0.1
3	0.05	37	1	0.1
4	1	37	1	1
5	1	27	0.1	0.1
6	0.05	37	0.1	0.1
7	0.05	37	0.1	1
8	0.05	27	1	1
9	1	37	0.1	1
10	1	37	0.1	0.1
11	0.05	37	1	1
12	0.05	27	0.1	1
13	1	27	1	1
14	1	27	0.1	1
15	0.05	27	0.1	0.1
16	0.05	27	1	0.1

Table 2. Experimental design matrix for determining critical factors that affect accumulation of a target protein.

2.11. Harvest the remainder of the cultures and store the cells at -20°C.

2.12. Prepare cell-free extracts as described in Task 1, step 2.3.

2.12.1 Whole cell extracts can be prepared from the samples taken in steps 2.4 and 2.10 as in Task 2, step 2.7.2.

2.13. Analyze the whole cell, soluble and insoluble fractions as in Task 2, step 2.7, by SDS-PAGE and if available functional or immunoblot assays.

3. Interpretation

3.1. Determine the amount of target protein accumulated in each of the different culture conditions.

3.1.1. To determine the approximate total amount of protein produced, analyze the whole cell extracts by SDS-PAGE and calculate the amount of protein using densitometry scans of the stained gel.

3.1.2. To determine the amount of target protein in the soluble fraction, prepare cell-free extracts and measure the amount of activity (by functional assay) or amount of protein (by SDS-PAGE). (Alternatively, a quantitative immunoblot could be used to determine the relative accumulation levels.)

3.2. Determine the factors which are having a major effect on protein accumulation.

Critical Factors that Affect Accumulation of a Target Protein					
Culture No.	**IPTG**	**Temp.**	**LB*Booster™**	**Augmedium™**	
1					
2					
3					
...					
...					
16					
	0	0	0	0	Sum_{High}
	0	0	0	0	Sum_{Low}
	0	0	0	0	Relative Effect

Table 3. Template for determining critical factors.

3.2.1. Prepare a spreadsheet with 16 rows corresponding to cultures numbered 1 to 16 and 4 columns corresponding to each of the factors tested (inducer concentration, temperature, LB*Booster concentration, Augmedium concentration) as shown in Table 3.

3.2.2. Enter the value (i.e., enzyme activity, mass, pixel density etc.) obtained for each culture into each cell in the row. For any given culture each factor will have the same value entered. (Note: numeric descriptors for qualitative assessments will also work, but with less accuracy.)

3.2.3. Calculate the sum of protein recovered for each factor when the factor was high in the solution. Sum_{High} (For example, for IPTG sum cultures 1,2, 4, 5, 9, 10, 13 and 14.)

3.2.4. Calculate the sum of protein recovered for each factor when the factor was low in the solution. Sum_{Low}. (For example, for IPTG sum cultures 3, 6, 7, 8, 11, 12, 15 and 16.)

3.2.5. Calculate the difference between the High and Low and divide by 8 for each factor. Relative Effect = $[Sum_{High} - Sum_{Low}] / 8$.

3.2.6. Compare the Relative Effect numbers obtained.

3.2.6.1. A positive number indicates a positive effect on protein production.

3.2.6.2. A negative number indicates no or an adverse effect on protein production.

3.2.6.3. The larger the positive number the greater the effect of the given factor.

3.2.7. Once the critical factor or factors have been identified, the culture conditions can be further optimized by examining the effect on protein accumulation of the identified factor. Step 4 below provides a suggested method.

3.2.8. Alternatively, if the production level is sufficient for the intended use of the target protein, the culture conditions yielding the highest level of production can be used for further work.

3.2.9. Other factors that can affect the level of protein accumulation, particularly soluble protein, include i) induction duration, ii) glycine levels, iii) non-ionic detergent levels, and iv) lactose versus IPTG (in $LacY^+$ strains only).

4. Optimization of the Culture Conditions: For the optimization of one factor only.

4.1. Prepare a 150 mL final volume culture as described in Task 3, steps 2.1-2.4.

4.2. Divide the culture into five 25 mL portions and treat as described for the factor being tested, using two levels above and two below the level found to give higher relative accumulation in Task 3.

4.3. Determine the conditions that yield the highest level of target protein using a functional assay, SDS-PAGE or immunoblot.

4.4. Refine as needed for each factor.

Supplemental Protocols

Supplemental Protocol 1: Rapid Transformation Protocol (Kit available: AthenaES™ No. 0156)

1. Materials:

1.1. 2x TSS (DO NOT store or use near flame, DMSO is flammable).

1.2. *E. coli* strains to be transformed.

1.3. Tryptic Soy Agar (TSA) plates or comparable non-antibiotic containing plate medium.

1.4. Sterile micro(centri)fuge tubes, 1.5mL (or other convenient tube).

1.5. LB medium.

1.6. SOC medium.

1.7. Antibiotic-containing plate medium.

2. Methods:

2.1. Streak strain(s) on non-antibiotic plate medium and incubate at 37°C overnight. (This protocol MUST be performed with fresh, overnight bacterial colonies.)

2.2. Dispense 0.1 mL LB medium into microfuge tubes using sterile technique.

2.3. Pick 4 colonies using a 1µL loop or sterile toothpick and resuspend in the 0.1 mL LB using sterile technique.

2.4. Add 0.1 mL 2x TSS (DO NOT FLAME) and mix well.

2.5. Incubate on ice for 15 minutes. Once chilled, do not allow the cells to warm above 14°C.

2.6. Add 100 ng plasmid DNA and incubate on ice for 20 min.

2.7. Heat shock in a 42°C water bath for 1 min.

2.8. Add 1 mL SOC medium using sterile technique.

2.9. Incubate at 37°C for 30 min.

2.10. Plate 0.1 mL of transformation mix on antibiotic-containing plate medium and incubate overnight at 37°C for 30 min.

2.11. Streak purify 2 or 3 colonies on appropriate antibiotic plates.

2.12. Prepare master cell bank(s) or cryostock(s) of new strain(s).

Tips of the Trade

Tip 1: Media Optimization Inducer

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 good 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 can not 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 Paste

Once the medium yielding the highest level of expression has been determined, the cell paste can be used to prepare a small-scale extract.

Tip 4: SDS-PAGE gels

Coomassie Blue stain should be sufficient to visualize the expression of a recombinant protein. 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 better resolution of individual polypeptide bands.

Tip 5: Alternative Techniques

Alternative techniques can be applied to the media screen analysis. Immunoblot or functional assays can be employed as appropriate. 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 the 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.

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.

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 PERK™ 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.

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 specifications. Notice for replacement must be received within 60 days of opening the product.

Ordering Information

To place an order:

Phone: 1-888-892-8408 Email: media@athenaes.com
Fax: 410-455-1155 Website: www.athenaes.com

Or visit our website to order through one of our international 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.

PERK™ Replacement Products Information

Catalog Number	Product	Size
0103	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
0123	LB Booster™	100 mL Stock
0124	LB Booster™	500 mL Stock
0125	Augmedium™	100 mL Stock
0126	Augmedium™	500 mL Stock