ACES™

ACES™ ■ Signal Sequence Kit

AthenaES™ Complete Expression System

The ACES™ Signal Sequence Kit provides six signal sequence variants that transport target proteins to the periplasmic space of *E. coli,* utilizing either the SEC, SRP or TAT secretion pathways and allowing for selection of the translocation pathway best suited for a target protein.

AthenaES®

Athena Enzyme Systems™
888-892-8408
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Application Manual
V 1.1

ACES™

AthenaES™ Complete Expression System

Signal Sequence Kit

Application Manual

V. 1.1

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ACES™ Signal Sequence Kit
Application Manual

Table of Contents

Introduction

E. coli is often the host strain of first choice for the production of recombinant proteins. Despite its long and successful history, several limitations remain when the desired protein cannot be expressed in a functional state. This is often due to the inherent properties of expressing heterologous proteins in the cytoplasm. One means of overcoming these impediments is to express the protein such that it is secreted into an environment that is more conducive to correct folding and where proteolytic degradation is minimized, such as the periplasm or extracellular matrix. Periplasmic or extracellular protein export has been exploited for the production of a number of recombinant proteins.[1] The extracellular accumulation of a target protein provides several advantages. These include:

- **A Simplified Downstream Purification Scheme**

E. coli does not naturally export a significant number or amount of proteins to the extracelluar matrix. Therefore, the level of host cell proteins as well as endotoxin and nucleic acids, contaminants that present considerable challenges to the purification of proteins destined for pharmaceutical use; would be significantly reduced.

- **Enhanced Biological Activity**

Export through the periplasmic space exposes proteins to a set of disulfide isomerases and foldases which facilitate correct protein folding as well as to an oxidizing environment which favors disulfide bridge formation.

- **Higher Product Stability and Solubility**

In addition to chaperones, there are fewer proteases in the periplasmic space and even fewer extracellular proteases.

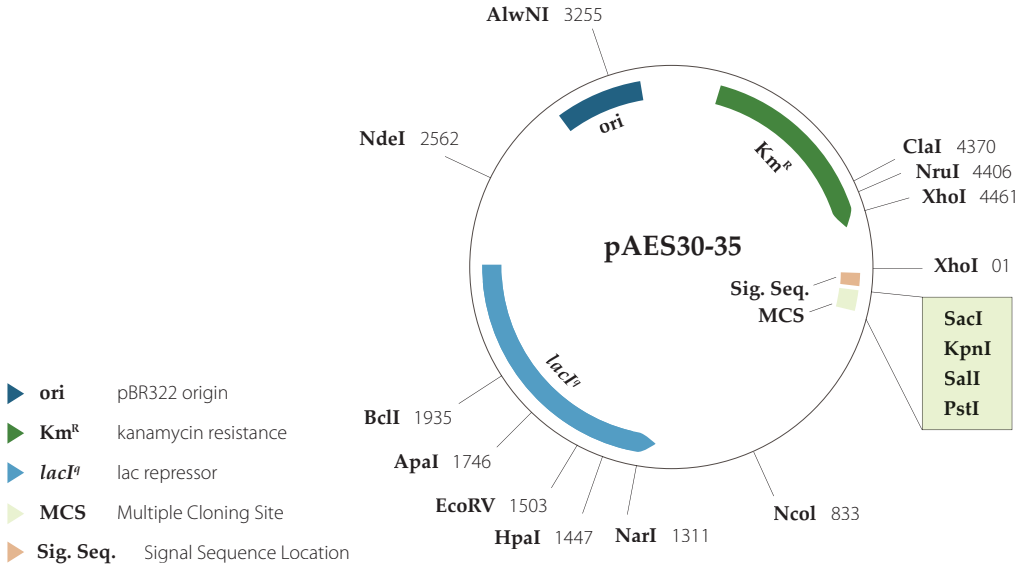
Protein secretion in *E. coli* and other Gram-negative bacteria is an extensively reviewed subject.[1] It is also a very complex process. The pAES30 series vectors utilize signal sequences that represent the three translocation pathways of the type II secretion process of *E. coli.*

The type II secretion mechanism is a two-step process. Protein translocation through the inner membrane is accomplished by way of one of three pathways: SecB-dependent (SEC), signal recognition particle (SRP) or twin-arginine translocation (TAT) (Fig. 1). Most proteins in *E. coli* are exported to the periplasm via one of these pathways with the larger majority using SEC. Extracellular secretion by a type II mechanism is via the main terminal branch pathway, which involves a poorly defined complex of 12-16 proteins that are not expressed under normal laboratory conditions.[2] Therefore, most of the protein exported by the type II systems remains resident in the periplasm, which is also where most secreted recombinant proteins accumulate.[3]

See the ACES™ Signal Sequence and YebF Protein Export Systems Technical Brief at www.athenaes.com for more detailed information about protein translocation in E. coli.

Principle of the Kit

Six signal sequences representing the three translocation pathways of *E. coli* (SRP, SEC, TAT) are fused to the N-terminus of the Target Protein facilitating its secretion to the periplasmic space. Translocation through the inner membrane causes removal of the signal sequence resulting in a Target Protein with its native N-terminus.[4] Once in the periplasmic space, the Target Protein can then be recovered by using any number of reagents that perforate the outer cell membrane, causing release of the Target Protein into the culture medium. Alternatively, cells can be harvested by centrifugation and a cold osmotic shock protocol *(See Supplemental Protocols)* used to extract the recombinant Target Protein from the periplasmic space.



pAES30	SRP	4,715 bp	
AAAAAGATTTGGCTGGCGCTGGCTGGTTTAGTTTTAGCGTTTAGCGCATCGGCG			
pAES31	Sec	4,721 bp	
AAAAAGACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCG			
pAES32	Sec	4,721 bp	
AAACAAAGCACTATTGCACTGGCACTCTTACCGTTACTGTTTACCCCTGTGACAAAAGCG			
pAES33	TAT	4,753 bp	
TCACTCAGTCGGCGTCAGTTCATTCAGGCATCGGGGATTGCACTTTGTGCAGGCGCTGTTCCACT-GAAGGCCAGCGCAGCAGATCTACTAGT			
pAES34	TAT	4,789 bp	
AACAATAACGATCTCTTTCAGGCATCACGTCGGCGTTTTCTGGCACAACTCGGCGGCTTAACCGTCGCCG-GTATGCTGGGTCCGTCATTGTTAACGCCGCGACGTGCGACGGCAGCAGATCTACTAGT			
pAES35	SRP	4,712 bp	
CGCGTACTGCTATTTTTACTTCTTTCCCTTTTCATGTTGCCGGCATTTTCG			

Figure 1. Plasmid map and signal sequences of pAES30 series vectors.

Kit Components

ACES™ Signal Sequence Kit Components		
Component	**Amount**	**Catalog Number**
pAES30	10 µg	0149-30
pAES31	10 µg	0149-31
pAES32	10 µg	0149-32
pAES33	10 µg	0149-33
pAES34	10 µg	0149-34
pAES35	10 µg	0149-35
LB Broth (Miller)	1 L Mix	0103
Turbo Broth™	1 L Mix	0104
Superior Broth™	1 L Mix	0105
Power Broth™	1 L Mix	0106
Hyper Broth™	1 L Mix	0107
Glucose M9Y	1 L Mix	0108
Glucose Nutrient Mix	21 g	0109
Augmedium™	25 mL Mix	0123
Inducer Solution A	1mL	0152
Inducer Solution B	100mL	0153
Secretion Enhancer Solution A	60mL	0154
Secretion Enhancer Solution B	60mL	0155
Reagents needed but not provided: Glycerol, Kanamycin, LB plate medium containing 50 µg/mL kanamycin		

Solution Information	
Component	**Reagent**
Inducer Solution A	1 M IPTG
Inducer Solution B	20% Lactose
Secretion Enhancer Solution A	10% Glycine
Secretion Enhancer Solution B	10% Triton X-100

Protocols

Preparation: 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 the Turbo Broth™ and Power Broth™ solutions.
3. Dispense desired volume into appropriate bottles or flasks. (We recommend 2 x 500 mL glass bottles.)
4. Autoclave at 121°C for 20 min. The autoclaved media without antibiotics are stable for at least 6 months at 4°C.
5. Dissolve the contents of the Glucose Nutrient Mix in 100 mL deionized water and filter sterilize using a 0.2 µm 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.

Preparation: Medium Supplements

1. **Augmedium™ (50x Solution)**
   - 1.1. Dissolve the contents of the Augmedium™ container in 25 mL deionized water.
   - 1.2. Filter sterilize using a 0.2 µm filter.
   - 1.3. Aliquot 5 mL portions into sterile 15 mL conical tubes and store at -20°C. The stock will be a 50x solution.
   - 1.4. For use, snap thaw at 37°C and store at 4°C for no more than five (5) days.
2. **Inducer Solution A** (1M IPTG)
   - 2.1. Use as is to a final concentration of 1 mM.
   - 2.2. Store at -20°C.
3. **Inducer Solution B** (20% Lactose)
   - 3.1. Make 20% stock solution by dissolving contents of packet into 100 mL of deionized water.

3.1. Filter sterilize using 0.2 µm filter.

3.2. Store at 4°C.

4. Secretion Enhancer Solution A (10% Glycine)

4.1. Make 10% stock solution by dissolving contents of packet into 60 mL deionized water.

4.2. Filter sterilize using 0.2 µm filter.

4.3. Store at 4°C.

5. Secretion Enhancer Solution B (10% Triton X-100)

5.1. Use as is to a final concentration of 1%.

5.2. Store at 4°C.

Task 1: Subclone the Desired Gene Sequences into any or all of the pAES30 series vectors

Use any of the restriction sites located downstream of the signal sequence (SS) site to insert the coding sequence for the Target Protein. Be sure to design the subcloning such that the amino acid sequence of the Target Protein is in-frame with the signal sequence. (This fusion construct will be referred to as "SS-Target Protein".)

Task 2: Express the SS-Target Protein Fusion

The protocol presented below is the basic method for producing proteins expressed using the Signal Sequence Vectors. Inducer Solution A or B can be used as the inducer. Inducer Solution A is used initially at a concentration of 1 mM and can be increased or decreased depending on the viability of the host strain and production levels of the Target Protein. Inducer Solution B can be used if the bacterial host has the appropriate genotype; i.e., the strain is wild-type for lacZY. Inducer B is used at a concentration of 2%. Induction proceeds for 3 hours to overnight, depending upon the time point, post-induction, that accumulates maximum Target Protein. Any rich medium can be employed. This kit contains several rich proprietary media formulated for the optimized expression of recombinant proteins in *E. coli.*

- **Basic Protocol for Task 2:** (For 25 mL, Adjust Volumes as Needed)

1. Introduce the plasmid construct from Step 1 into the desired production strain.

    1.1. Prepare an isogenic parent strain for each fusion construct by transforming the pAES30 series vector used in the fusion construction into the host strain of choice.

    1.2. Supplemental Protocol 1 gives a protocol for the rapid transformation of any bacterial vector into any *E. coli* strain.

    1.3. Streak purify two to eight SS-Target Protein transformants and verify the integrity of the plasmid construct.

    1.4. Store all strains accordingly.

2. Use a single colony to inoculate 2x 2 mL starter cultures supplemented with 50 µg/ml kanamycin - the isogenic parent and SS-Target Protein construct.

    2.1. Turbo Broth™ (AthenaES™ Cat. No. 0104) is recommended but any rich medium will suffice.

    2.2. Incubate at 37°C overnight.

3. Inoculate 2x 25 mL of medium (supplemented with 50 µg/ml kanamycin) with the overnight cultures and incubate at 37°C until the OD_{600} reaches 0.8 - 1.0.

3.1. The use of baffle bottomed flasks is strongly recommended to provide sufficient aeration.

3.2. Turbo Broth™ is recommended but any rich medium will suffice.

4. Remove 1 mL culture as pre-induction/uninduced samples to 1.5 mL microfuge tubes.

4.1. In plastic cuvettes, dilute 100 µL of each culture in 900 µL of water and measure the absorbance at OD_{600} in a spectrophotometer. Multiply the absorbancy by 10 to calculate the culture density. This number should be between 0.5 and 3.0.

4.2. Harvest cells by microfuging for 2 min. Aspirate all media off the cell pellets.

4.3. Suspend each pellet in water to give 10 OD units/mL. (For example, if the culture density was 1.0, resuspend the pellet in 100 µL water.)

4.4. Reserve or immediately prepare samples for SDS-PAGE analysis. (Step 7 below).

5. Induce expression by adding 25 µl Inducer Solution A (IPTG) (to 1 mM), or 2.5 mL Inducer Solution B (Lactose) (to 2%). *(Tip 1)*

5.1. Note: Inducer Solution B can only be used if the host strain is wild-type for lacZ and lacY.

6. Continue to incubate at 37°C, removing 1mL samples at T1, T2, T3 and T20-24 post-induction.

Dilute samples to 10 OD/mL (as described above in step 4.1.)

7. Prepare samples for SDS-PAGE as follows:

7.1. Mix 35 µl of water, 10ul 5x SDS-PAGE loading dye and 5 µl of each 10 OD/ml culture sample (including the pre-induction/T0 sample) in a 0.5 ml microfuge tube.

7.2. Incubate at 100°C for 5 min.

7.3. Load 10 µl of each sample into the wells of a standard SDS-PAGE polyacrylamide gel. (Tris-glycine gradient gels are suitable.)

7.4. Load 10 µl of protein markers into outside wells.

7.5. Electrophorese and stain the gel with Coomassie blue, colloidal blue or silver stain *(Tip 4)*

8. **Interpretation**

8.1. Compare lanes with and without IPTG to determine differential expression of the Target Protein. Also, compare the lanes containing the Target Protein vs. the isogenic parent lanes to verify expression of the recombinant protein. An observable increase in intensity of a protein band of the expected molecular mass from the uninduced culture to the induced culture (and from the Target Protein to the isogenic parent culture) is indicative of positive expression.

8.2. Compare relative staining intensity of the recombinant protein between post-induction time points. The time point with the highest level of production will determine the induction regimen for future experiments (unless other parameters, like a strain screen or media optimization screen [*see Supplemental Protocols*], are investigated).

Task 3: Screen for Target Protein Expression

Screening for Target Protein expression can be done by two methods. The first is by using the "cold osmotic shock" method *(see Supplemental Protocol 6)* to release Target Protein from the periplasm of the cells following an induction experiment. The second is to employ reagents that perturb the outer cell membrane such that the Target Protein accumulating in the periplasmic space is released to the culture medium during an induction experiment. This second method, which involves reagents provided in the kit, will be described here.

1. Set up 4 x 2 ml starter cultures of the strains containing the SS-Target Protein and 1 x 2 ml pAES30 series isogenic parent vector.

    1.1. Use a rich medium such as Turbo Broth™ (AthenaES™) or Terrific Broth rather than LB medium. Add kanamycin to 50 µg/ml.

    1.2. Incubate overnight at 37°C with shaking.

2. Inoculate 4 x 25 ml rich medium (containing 50ug/ml kanamycin) with each of the Target Protein overnight starter cultures.

    2.1. Inoculate 1 x 25 ml rich medium with the overnight pAES30 isogenic parent culture.

    2.2. Incubate at 37°C with shaking until the OD_{600} reaches 0.8 - 1.0.

3. Remove 5 ml pre-induction (T0) samples to a centrifugable tube. Treat the culture sample as in 4.3 below.

4. Induce expression by adding the inducer reagent of choice from Task 2.

    4.1. To one Target Protein culture also add Secretion Enhancer Solution A to 1.0% (*See Medium Supplements, page 6*); to another culture add Secretion Enhancer Solution B to 1.0%; to the third culture add both Secretion Enhancer Solutions; add nothing to the fourth culture (as the 'no additive' control).

    4.2. Continue to incubate at 37°C, removing 5 mL samples at T3-6hrs and T20-24hrs, post-induction (or at those times at which maximum expression of the Target Protein was observed in Task 2).

    4.3. As in Task 2, dilute a portion of the 5 ml samples to 10 OD/ml to be used for immunoblot and/or SDS PAGE analysis. Pellet the cells from the remaining 5 ml of diluted sample by centrifuging at 5,000xg for 20min. Reserve the culture supernatants for analysis.

    4.4. Store the cell pellets at 4°C for later analysis, if desired.

5. Analyze the culture supernatants (and the cell pellets, if desired) by immunoblot (anti-Target Protein antibodies), ELISA or functional assay. Analysis by SDS-PAGE gel can be done, but note that the accumulation of the Target Protein in a non-optimized expression regimen may be below the detection limits of SDS-PAGE.

   5.4. Use the culture conditions that produced the most Target Protein in the supernatant samples for subsequent experiments.

   5.5. Ideally, further optimization should be peformed by 1) screening for the growth medium that yields the most Target Protein *(Supplemental Protocol 2)*, 2) screening for the bacterial host strain that yields the most Target Protein *(Supplemental Protocol 3)*, and/or 3) titering reagents or using supplemental reagents (e.g. Augmedium) in order to further optimize the expression regimen *(Supplemental Protocol 4)*.

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 microcentrifuge tubes, 1.5 mL (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).

Supplemental Protocol 2: Media Optimization Protocol

1. Materials

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

1.2. Reagents for Immunoblot, Functional Assay or SDS-PAGE.

2. Methods

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

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

2.3. Remove a 1 ml sample ("pre-induction"), harvest the cells and store the culture medium at 4°C for later analysis. Save the cell pellet if screening for overall Target Protein yields as in Task 2 and/or the culture medium if screening for secreted Target Protein yields as in Task 3.

2.3.1. Add the Inducer Solution A (final IPTG concentration of 1mM) or 2.5mL of Inducer Solution B (final lactose concentration of 2%) and continue incubating for T3 to T22 hours (depending on post-induction time points that yielded the most Target Protein from Task 2 or Task 3). If following the protocol in Task 3, add the Secretion Enhancer Solutions as stated there.

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

2.5. Separate the cells from the remaining culture medium by centrifugation at 5,000 xg for 20 minutes and store the cell pellets and supernatants at 4°C. Determine the relative level of Target Protein in the pre-induction and post-induction samples for each culture condition using: 1) SDS-PAGE, if analyzing expression in whole cells (i.e., without having added Secretion Enhancer reagents, as in Task 2 or 2) an immunoblot (using anti-Target Protein antibodies) or a functional assay, if analyzing expression in the culture medium, as in Task 3. Select the medium yielding the highest level of Target Protein accumulation for further work.

Supplemental Protocol 3: Strain Optimization Protocol

1. Introduce the plasmid construct from Step 1 into each of three to six host strains. The most commonly used strains for the production of recombinant proteins can be used. See Supplemental Protocol 1 for a simple rapid transformation protocol. It is recommended that the parent pAES30 series plasmid(s) be introduced into each strain to serve as an (isogenic parent) control(s) in expression experiments.

2. Follow the expression protocol in either Task 2 or Task 3.

   2.1. Select the strain(s) yielding the highest level of expression of the Target Protein.

Supplemental Protocol 4: Culture Conditions Optimization Protocol

1. Determining the optimal culture conditions is an iterative process. First, a two-level fractional factorial experimental design is used to identify the critical factors affecting the accumulation of the Target Protein.[5] Table 1 lists the conditions to be tested. Augmedium™ (supplied in the kit) can be used to augment Target Protein production. Use an immunoassay or functional assay to quantify the level of expression and select the conditions that yield the highest level of production. Further optimization is then done by modifying those parameters identified as critical to accumulation of the Target Protein *(Supplemental Protocol 5)*.

Critical Factors that Affect Accumulation of the Target Protein						
Culture	**IPTG (mM)**	**Lactose (%)**	**Glycine (%)**	**Triton X-100 (%)**	**Augmedium™ (x)**	**Temp.**
1	1	0	1	0	0	37°C
2	0.05	2	1	0	0	27°C
3	1	2	1	1	1	37°C
4	1	0	0	0	1	27°C
5	1	2	0	0	0	37°C
6	1	2	1	0	1	27°C
7	0.05	0	0	0	0	27°C
8	1	0	0	1	1	37°C
9	0.05	2	1	1	0	37°C
10	1	0	1	1	0	27°C
11	0.05	2	0	1	1	27°C
12	1	2	0	1	0	27°C
13	0.05	0	0	1	0	37°C
14	0.05	0	1	0	1	37°C
15	0.05	0	1	1	1	27°C
16	0.05	2	0	0	1	37°C

Table 1. Experimental design matrix for determining critical factors that affect accumulation of a target protein.

Supplemental Protocol 5: Determining Critical Factors for Expression Protocol

1. Prepare a spreadsheet with 16 rows corresponding to culture conditions 1 through 16 and 6 columns corresponding to each of the factors tested and the solutions as shown in the figure below.

2. Enter the value (i.e., enzyme activity, mass, etc.) obtained for each culture condition into the respective cell in the row. For any given condition each factor will have the same value entered.

    2.1. Note: Numeric descriptors for qualitative assessments will also work, but with less accuracy.

3. Calculate the sum of protein produced for each factor when the factor was present in the solution. SumPresent

    3.1. Note: For IPTG consider the 1 mM level "Present."

4. Calculate the sum of protein recovered for each factor when the factor was absent from the solution. SumAbsent

    4.1. Note: For IPTG consider the 0.05 mM level "Absent."

5. Calculate the difference between the Present and Absent and divide by 8 for each factor. Relative Effect = SumPresent - SumAbsent / 8.

6. Compare the Relative Effect numbers obtained

    6.1. A positive number indicates a positive effect on accumulation.

    6.2. A negative number indicates no effect on accumulation.

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

Critical Factors that Affect Accumulation of the Target Protein						
Culture	**IPTG (mM)**	**Lactose (%)**	**Glycine (%)**	**Triton X-100 (%)**	**Augmedium™ (x)**	**Temp. (°C)**
1						
2						
.						
.						
.						
15						
16						
$Sum_{Present}$						
Sum_{Absent}						
Rel. Effect						

Table 2. Example analysis table used to determine the factors critical to protein accumulation.

Supplemental Protocol 6: Cold Osmotic Shock Protocol [6]

- For releasing the Target Protein form the periplasmic space.

1. Materials

1.1. 30 mM Tris-Cl/20% sucrose solution, pH 8.0

1.2. 0.5 M EDTA, pH 8.0

1.3. 0.5 M sodium phosphate buffer, pH 7.2

1.4. 5 mM Magnesium sulfate

2. Methods

2.1. Perform induction experiment as in Task 2 saving cell pellets from T0 and all of the post-induction time points. (DO NOT FREEZE these cell pellets. Process immediately or store at 4°C.)

2.2. Resuspend the cells in 10ml of 30mM Tris-Cl/20% sucrose, pH 8.0 (0.08ml for each milligram of cellular wet weight).

2.3. Add 20 µl of 0.5M EDTA, pH 8.0 and incubate 5 to 10 min at room temperature with shaking or stirring.

2.4. Centrifuge 10 min at 8000 rpm (10,000 x g) at 4°C. Remove all of the supernatant and resuspend the pellet in 10 ml ice-cold 5 mM $MgSO_4$

2.5. Shake or stir 10 min in an ice bath.

2.6. Centrfuge again as in Step 4. Save the supernatant, which is the cold osmotic shock fluid.

2.7. Add 200 µl of 0.5 sodium phosphate buffer, pH 7.2

2.8. Analyze samples by SDS-PAGE, immunoblot or functional assay.

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 preliminary expression experiments and for 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.

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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 ACES™ Signal Sequence Kit and Vectors were designed and are sold for research use only. None of the kit components should 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.

The plasmids and other components contained in the ACES™ Signal Sequence Kit are covered under international patents and patent applications exclusively licensed by the University of Alberta to Athena Environmental Sciences, Inc. The use of these reagents requires a license.

- Academic and Not-for-Profit entities are required to execute a non-commercial use license. This agreement is a non-fee bearing license which grants the user the rights to use the plasmid and other kit components for research purposes only and restricts the user from disseminating the plasmids to other researchers without the expressed written consent of AthenaES™.

- Commercial users are required to execute a commercial evaluation license agreement. The agreement is a non-fee bearing license which grants to the user the right to use the plasmid and other kit components for research purposes only for the period of one (1) year after which a commercial use license is required.

Copies are both types of license agreements are available at www.athenaes.com.

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: aesinfo@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.

ACES™ Accessory Products Information

Catalog Number	Product	Size
0149-25	pAES25	10µg
0149-30	pAES30	10µg
0149-31	pAES31	10µg
0149-32	pAES32	10µg
0149-33	pAES33	10µg
0149-34	pAES34	10µg
0149-35	pAES35	10µg
0149-40	pAES40	10µg
0150-1	Primer A	250pmoles
0150-2	Primer B	250pmoles
0152-1	Inducer Solution A	1mL
0152-5	Inducer Solution A	5 x 1mL
0153	Inducer Solution B	500mL
0154	Secretion Enhancer A	500mL
0155	Secretion Enhancer B	500mL
0156	Rapid Transformation Kit	1 kit
0157	2x TSS	5 x 1 mL
0313-1	Anti-YebF Antisera	0.5mL