

## ExpressMax™ Fermentation Media

Catalog Numbers: 0136 - 0147

### Product Description

ExpressMax™ fermentation media aid in the production of recombinant proteins. The series is comprised of eight pre-formulated media, designed to cover a range of possible expression conditions and maximize the production of recombinant proteins. The ExpressMax™ basal salts are inorganic nutrients optimized to generate the highest possible growth rate of *E. coli*, resulting in maximal production of recombinant proteins. The screening kit is intended to aid in formulation development by supplying the basal salts/trace elements mix along with the carbon source (glucose) and nitrogen sources (yeast extract, soy protein hydrolysate and Atholate™) which can be combined in different variations to identify the composition that gives the highest production of the target protein.

### Formula Components

Formula	Components (g/L)				
	Fermentation Salts & Trace Metals Mix	Yeast Extract	Atholate™	Soy Hydrolysate	Glucose
1	38.27	0.0	0.0	0.0	10
2	38.27	5.0	0.0	0.0	10
3	38.27	0.0	5.0	0.0	10
4	38.27	0.0	0.0	5.0	10
5	38.27	5.0	5.0	0.0	10
6	38.27	5.0	0.0	5.0	10
7	38.27	0.0	5.0	5.0	10
8	38.27	5.0	5.0	5.0	10

### Formulations

Components (mL)	Formula							
	1	2	3	4	5	6	7	8
(5x) Fermentation Salts & Trace Metals Mix	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
(5x) Yeast Extract	0.00	5.00	0.00	0.00	5.00	5.00	0.00	5.00
(5x) Atholate™	0.00	0.00	5.00	0.00	5.00	0.00	5.00	5.00
(5x) Soy Hydrolysate	0.00	0.00	0.00	5.00	0.00	5.00	5.00	5.00
(20x) Glucose Nutrient Mix	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Sterile Water	18.75	13.75	13.75	13.75	8.75	8.75	8.75	3.75

### Instructions for Use

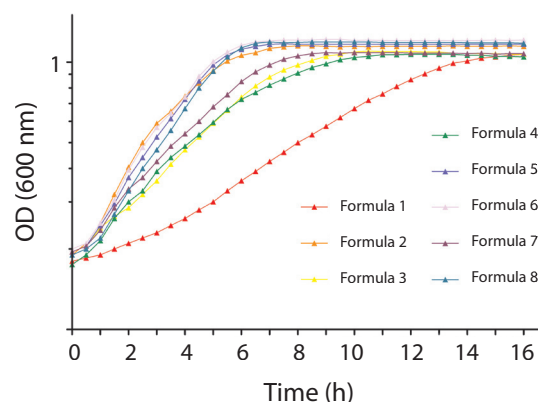
- Materials** - Kit contains enough sterile liquid reagents to conduct one 8-flask screening experiment
  - LB Broth
  - 5x Fermentation Salts & Trace Metals Mix (191.36 g/L)
  - 5x Yeast Extract (25 g/L)
  - 5x Atholate™ (25 g/L)

See second page for continued instructions.



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**Growth Curves of an *E. coli* HMS174 Strain  
 ExpressMax™ Media Formulation Screen**



**Figure 1.** An overnight culture was used to inoculate 0.5 ml of each medium in duplicate wells of a 24-well culture dish to give an OD<sub>600</sub> of 0.2. The plate was incubated with shaking at 37°C and the absorbance monitored at 30 minute intervals.

### Fermentation Salts & Trace Metals Mix (5x)

Component	g/L
KH <sub>2</sub> PO <sub>4</sub>	30.0
K <sub>2</sub> HPO <sub>4</sub>	95.0
Na <sub>2</sub> HPO <sub>4</sub>	45.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	12.5
Citric Acid	8.5
FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub>	0.250
ZnSO <sub>4</sub> *7 H <sub>2</sub> O	0.025
CoCl <sub>2</sub> *6 H <sub>2</sub> O	0.025
Na <sub>2</sub> MO <sub>4</sub> *2 H <sub>2</sub> O	0.025
CuSO <sub>4</sub> *5 H <sub>2</sub> O	0.025
H <sub>3</sub> BO <sub>3</sub>	0.010

### 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.

- 1.5. 5x Soy Hydrolysate (25 g/L)
- 1.6. 20x Glucose Nutrient Mix (200 g/L)
- 1.7. 250 mL baffle bottomed flasks (8)
- 1.8. Wash Buffer: 40 mM sodium phosphate pH 7.5, 150 mM NaCl
- 1.9. 5x SDS-PAGE Loading Dye: 124 mM Tris-Cl, 1.28 M 2-Mercaptoethanol, 0.1 M glycerol, 0.14 M SDS (sodium dodecyl sulfate), 2.9 mM bromophenol blue
- 1.10. Tris-Glycine 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 baffle bottomed shake flask. Incubate at 37°C overnight.
- 2.2. Prepare eight 250 mL baffle bottomed flasks containing 25 mL of each of the formulas as per the table above.
- 2.3. Inoculate 25 mL of each of the eight media with 1 mL of the overnight culture. Incubate the cultures at 37°C until the OD<sub>600</sub> reaches 0.6.
- 2.4. Remove a 1 mL (“pre-induction”) sample, harvest the cells in a microfuge tube, and suspend the cells in water to a density of 10 OD/ml. Store at -80°C.
- 2.5. Add inducer and continue incubating for 3 hours.
- 2.6. Remove a 1 mL (“post-induction”) sample and process as in step 2.4.
- 2.7. Harvest the remainder of the cultures, wash the pellets with 10 mL of wash buffer, reharvest the cells, discarding the supernatant/wash. Determine the mass of the cell pellet and store the cell pellets at -80°C.
- 2.8. Analyze for expression of the target protein as follows:
  - 2.8.1. To determine protein production per mL of culture:
    - 2.8.1.1. Thaw the resuspended cells from pre- and post-induction at 37°C.
    - 2.8.1.2. Mix 5 µL of each cell suspension with 35 µL water and 10 µL 5x SDS-PAGE loading dye. Heat at 100°C for 5 minutes and load 10 µL per lane of polyacrylamide gel.
  - 2.8.2. Stain the gel with Coomassie Blue, colloidal Coomassie Blue or Silver stain as necessary.

## 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.
  - 3.1.1. Compare the level of target protein from cells grown in each of the eight formulations of ExpressMax™ media. Select the medium which produces the highest level of target protein per mL of culture.
- 3.2. Once the appropriate nitrogen sources have been identified, further optimization should be performed to determine the proper concentration of the nitrogen source(s) for the protein of interest.

## Individual Reagent Information

All kit components (i.e., Atholate™, Yeast Extract, Basal Salts, Glucose Nutrient Mix, and Soy Hydrolysate), as well as any of the 8 final formulations are available in larger quantities from AthenaES™.

## Reorder Information

Catalog No.	Product	Amount
0136	ExpressMax™ Screening Kit	1 Kit
0137	ExpressMax™ Formula 1	500g
0138	ExpressMax™ Formula 2	500g
0139	ExpressMax™ Formula 3	500g
0140	ExpressMax™ Formula 4	500g
0141	ExpressMax™ Formula 5	500g
0142	ExpressMax™ Formula 6	500g
0143	ExpressMax™ Formula 7	500g
0144	ExpressMax™ Formula 8	500g
0145	Basal Salts	500g
0146	Yeast Extract	500g
0147	Soy Protein Hydrolysate	500g

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