invitrogen
by life technologies

MagicMedia™ *E. coli* Expression Medium

Description

MagicMedia™ *E. coli* Expression Medium promotes high-yield growth of *E. coli* and high-level expression of T7-regulated heterologous proteins without time consuming steps such as monitoring optical density (OD) or adding induction components such as IPTG. Unlike traditional LB-IPTG induction systems, MagicMedia™ medium allows regulated protein expression in any expression system that is solely inducible by IPTG from *E. coli* strains containing a functional lac operon. The protein expression starts automatically after inoculation with a single colony or starter culture, and subsequent overnight growth under standard conditions. MagicMedia™ *E. coli* Expression Medium is supplied in two convenient formats. MagicMedia™ Component A is available as a dry powder or in a pre-sterilized, ready-to-use liquid. Before growth and induction of bacteria, combine MagicMedia™ Components A and B, add antibiotic of choice, and inoculate.

Product	1 L Foil Pouch™ (Cat no. K6810)	5 × 1 L Foil Pouch™ (Catalog no. K6815)	1 L liquid (Catalog no. K6803)	Storage
MagicMedia™ Component A	40 g	5 × 40 g	950 mL	Room temperature
MagicMedia™ Component B	50 mL	5 × 50 mL	50 mL	

Product use

For Research Use Only. Not for use in diagnostic procedures.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Vectors and bacterial strains

MagicMedia™ *E. coli* Expression Medium is designed for T7-regulated protein expression systems. T7-regulated expression vectors (such as pET) are suitable for cloning your gene of interest. *E. coli* expression strains that contain a functional lac operon (including lacY and lacZ) such as BL21(DE3), BL21 Star (DE3), and BL21(DE3)/pLysS strains can be used with MagicMedia™ *E. coli* Expression Medium. **Important:** *E. coli* cloning strains such as DH5 or TOP10 cannot be used for induced expression.

Reconstitute MagicMedia™ Component A (dry powder)

1. Add the entire pouch of MagicMedia™ Component A to 950 mL ultrapure water in an autoclavable flask.
2. Autoclave on liquid cycle for 20 minutes. Cool media to 37°C.
3. Add selective antibiotic of choice (e.g. 100 µg/mL ampicillin) depending on the resistance gene in your vector. Use sterile technique during addition of antibiotic. Proceed to **Add MagicMedia™ Component B**, below.
   Note: Media color may be slightly darker after autoclaving. This is normal and will not affect product performance.

MagicMedia™ Component A Ready-To-Use Liquid (supplied as a sterile solution)

1. Using sterile technique, add a selective antibiotic of choice (e.g. 100 µg/mL ampicillin) depending on the resistance gene in your vector.
2. Proceed to **Add MagicMedia™ Component B**, below.

Add MagicMedia™ Component B

To prepare complete medium immediately before inoculation, add the entire volume of MagicMedia™ Component B to MagicMedia™ Component A (containing antibiotic of choice) using sterile technique and mix by swirling the flask. Store unused complete medium at 4°C for one month.

Note: MagicMedia™ Component B may have a slight precipitate, which will not affect product performance. If precipitate is observed, warm the bottle to 37°C to redissolve and add to media as described above.

Culture vessel size and incubation conditions

Optimal culture aeration during incubation is critical to achieve high-density growth. MagicMedia™ expression cultures must be incubated with vigorous shaking at 300 rpm in an appropriate vessel. For small-scale expression cultures, add 1 mL complete MagicMedia™ medium to a sterile 15 mL conical tube. For larger volumes in non-baffled flasks, use 10% flask volume of complete MagicMedia™ medium. For baffled flasks, use 20% flask volume of complete MagicMedia™ medium. Incubation with shaking at 37°C is generally recommended for maximum growth of MagicMedia™ expression cultures. Depending on your protein, incubation with shaking at 30°C may improve protein folding and solubility.

Starter culture

A starter culture is recommended for MagicMedia™ expression culture volumes >100 mL. To make a starter culture, inoculate 1/20 of the final culture vessel volume of LB media + selective antibiotic with your colony and grow overnight with shaking at 37°C. For expression culture volumes of <100 mL, directly inoculate MagicMedia™ medium with the colony.

Inoculation protocol

1. Add complete MagicMedia™ medium to sterile tubes or flasks according to the culture vessel size recommendations above.
2. Use sterile technique to inoculate the colony directly into the medium. Be sure to patch the colony onto a separate selective plate if needed. If you are using a starter culture, add the entire volume to MagicMedia™ medium using aseptic technique.
3. Cap tube or flask and secure in incubator.
4. Incubate at 30°C or 37°C with vigorous shaking (300 rpm) for 18–24 hours.

Dual temperature protocol

Use the dual temperature protocol for protein expression at temperatures lower than 30ºC. Protein expression at 18ºC or 25ºC is used for specific applications related to protein solubility and toxicity.

Day 1:

1. Inoculate 2–10 mL LB + antibiotic with an expression colony and incubate overnight at 30°C with vigorous shaking (300rpm).
2. Place appropriate amount of complete MagicMedia™ medium + antibiotic at 30°C to pre-warm it overnight.

Day 2:

1. Inoculate pre-warmed, complete MagicMedia™ medium + antibiotic with overnight seed culture at a 1:40 dilution.
2. Incubate culture at 30°C with vigorous shaking (300 rpm) for 6–7 hours or until O.D. 600 > 6.0.
3. Transfer culture to an incubator set at either 18°C or 25°C with vigorous shaking (300 rpm) and incubate for 24–36 hours.

Days 3–4:

Harvest culture and analyze results.

Optimizing Lysis Conditions:

Under the growth conditions specified above, *E. coli* cultures usually grow to > 3× density in MagicMedia™ medium compared to traditional medium. To ensure efficient bacterial lysis using your method of choice, you will need to estimate the amount of bacteria in your expression culture. Dilute an aliquot of the expression culture 1:20 in water and take an O.D. 600 reading using a spectrophotometer, and optimize your lysis conditions accordingly.

Related Products

Product	Cat. no.
Champion™ pET300/NT-DEST and pET301/CT-DEST Gateway® Vector Kit (1 kit)	K6300-01
Champion™ pET302/NT-His and pET303/CT-His Vector Kit (1 kit)	K6302-03
pET101 D-TOPO® Expression Kit (1 kit)	K101-01
pET100 D-TOPO® Expression Kit	K100-01
Champion™ pET SUMO Expression Kit	K300-01
pTrcHis2 A, B, & C	V365-20
His-Patch ThioFusion™ Expression System	K360-01
pRSET T7 Expression Vector	V351-20
BL21(DE3) Chem. Competent Cells	C6000-03
OneShot® BL21 Star™(DE3) Chem. Competent Cells	C6010-03
OneShot® BL21(DE3) pLysS Chem. Competent Cells	C6060-03

Troubleshooting

Problem	Cause	Solution
Low or no protein yield	Incompatible expression host	Use *E. coli* strains with a lac operon and T7 polymerase: BL21(DE3), BL21 Star™(DE3) or BL21(DE3) pLysS.
	Vector has no T7 or IPTG-inducible promoter	Use an expression vector with the T7 lac promoter, such as Champion™ pET or an IPTG inducible promoter.
	Culture has not reached stationary phase	• Let culture grow for up to 24 hours. • Make sure culture is properly aerated. Use baffled flasks and a 5:1 ratio of shake flask volume to culture volume for ideal growth. • Use the correct antibiotic selection, depending on the resistance gene present in your vector. • Use freshly-transformed colonies for inoculation. • Use a starter culture for expressions >100 mL, to avoid shock-induced lag phase growth caused by introducing a single colony to a large media volume.
	N- or C-terminal tags or fusions interfere with protein expression	Reclone gene of interest into a different vector and re-express protein in MagicMedia™ *E. coli* Expression Medium
	Protein is toxic to cells	Try the dual temperature protocol, above.

Explanation of Symbols and Warnings

LOT	REF		Read SDS	
Batch Code	Catalog number	Manufacturer	Read Safety Data Sheet	Use By:

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**.
For further assistance, email **techsupport@lifetech.com**

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