

# MagicMedia™ *E. coli* Expression Medium

**Cat. No. K6801**

**Size: 1 L SoluPouch™**

**Cat. No. K6802**

**Size: 5 x 1 L SoluPouch™**

**Cat. No. K6803**

**Size: 1 L liquid**

## Description

MagicMedia™ *E. coli* Expression Medium promotes high yield growth of *E. coli* and high-level expression of T7 regulated heterologous protein expression without time consuming steps such as monitoring optical density (OD) or adding induction components such as IPTG. Unlike traditional LB-IPTG induction systems, MagicMedia™ allows regulated expression of the T7 promoter system and allows high levels of protein expression from *E. coli* strains containing a *lac* operon 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. Component A is supplied dry in a convenient, fast-dissolving, water-soluble pouch (SoluPouch™), which is added to water and autoclaved. MagicMedia™ is also supplied in a pre-sterilized, ready-to-use liquid. Before growth and induction of bacteria, simply combine MagicMedia™ Components A and B, selective antibiotic of choice and inoculate.

## Components

|             | <u>1 L SoluPouch™</u> | <u>5 x 1 L SoluPouch™</u> | <u>1 L liquid</u> |
|-------------|-----------------------|---------------------------|-------------------|
| MagicMedia™ |                       |                           |                   |
| Component A | 40 g                  | 5 x 40 g                  | 950 ml            |
| MagicMedia™ |                       |                           |                   |
| Component B | 50 ml                 | 5 x 50 ml                 | 50 ml             |

## Storage

Store MagicMedia™ *E. coli* Expression Medium Component A SoluPouch™ and Component B at RT for up to 6 months. Store MagicMedia™ *E. coli* Expression Medium Component A liquid and Component B at 4°C for up to 6 months.

Part no. K6800.pps

Rev. date: 21 July 2006

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

For technical support, contact [tech\\_service@invitrogen.com](mailto:tech_service@invitrogen.com).

## Vectors and Bacteria Strains

MagicMedia™ 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™. See page 4 for ordering information.

**Important:** *E. coli* cloning strains such as DH5 $\alpha$  or TOP10 cannot be used for induced expression.

## Reconstituting MagicMedia™ Component A SoluPouch™

If you are using MagicMedia™ Component A SoluPouch™, **do not open the pouch**. Add the entire pouch to 950 ml ultrapure water in an autoclavable flask.

Autoclave on liquid cycle for 20 minutes. Cool media to 37°C and add selective antibiotic of choice (*e.g.* 100  $\mu$ g/ml ampicillin) depending on the resistance gene in your vector. Use sterile technique during addition of antibiotic. Proceed to **Adding 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

MagicMedia™ Component A ready-to-use liquid is supplied as a sterile solution. Add a selective antibiotic of choice (*e.g.* 100  $\mu$ g/ml ampicillin) depending on the resistance gene in your vector. Use sterile technique during addition of antibiotic. Proceed to **Adding MagicMedia™ Component B**, below.

## Adding MagicMedia™ Component B

To make complete media 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. Once combined, store unused media at 4°C for up to 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™ to a sterile 15 ml conical tube. For larger volumes in non-baffled flasks, use 10% flask volume of complete MagicMedia™. For baffled flasks, use 20% flask volume of complete MagicMedia™.

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, perform direct inoculation of MagicMedia™ with the colony.

## **Inoculation Procedure**

1. Add complete MagicMedia™ to sterile tubes or flasks according to the culture vessel size recommendations above.
2. Using sterile technique, inoculate the colony directly into the media. 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™ 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.

## **Next Steps**

Under the growth conditions specified above, *E. coli* usually grow to > 3x density in MagicMedia™ than traditional media. 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 OD<sub>600</sub> reading using a spectrophotometer, and optimize your lysis conditions accordingly.

## Quality Control

MagicMedia™ Components A and B are individually tested for pH, conductivity, sterility, color, and clarity. MagicMedia™ Components A and B together are tested for *E. coli* growth and functional protein expression.

## Additional Products

| Product                                                         | Amount     | Catalog 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    |
| BL21(DE3) Chem. Competent Cells                                 | 20 x 50 µl | C6000-03    |
| OneShot® BL21 Star™ (DE3) Chem. Competent Cells                 | 20 x 50 µl | C6010-03    |
| OneShot® BL21(DE3) pLysS Chem. Competent Cells                  | 20 x 50 µl | C6060-03    |

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