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by life technologies™

BL21 Star™(DE3) One Shot® BL21 Star™(DE3)pLysS One Shot® Chemically Competent Cells

Catalog nos. C6010-03, C6020-03

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User Manual

Table of Contents

About the Kit

Available Kits

The table below lists the One Shot® chemically competent cell kits covered by this manual. The transformation efficiency is calculated as number of transformants per µg of pUC19 plasmid DNA.

Item	Reactions	Transformation Efficiency	Catalog no.
BL21 Star™(DE3)	20	1×10^8 cfu/µg	C6010-03
BL21 Star™(DE3)pLysS	20	1×10^8 cfu/µg	C6020-03

Shipping/ Storage

Each One Shot® kit is shipped on dry ice. Upon receipt, store at –80°C.

Kit Contents

The table below describes the items included in each of the One Shot® chemically competent *E. coli* kits described above.

Store at –80°C.

Item	Composition	Amount
SOC Medium (store at room temperature or 4°C)	2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose	6 mL
Chemically competent cells	—	21 × 50 µL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µL

Continued on next page

About the Kit, Continued

Genotypes

BL21 Star™(DE3): F- *ompT hsdS*$_B$ ($r_B^-m_B^-$) *gal dcm rne131* (DE3)

BL21 Star™(DE3)pLysS: F- *ompT hsdS*$_B$ ($r_B^-m_B^-$) *gal dcm rne131* (DE3) pLysS (Cam^R)

The DE3 designation indicate the strains contain the DE3 lysogen that carries the gene for T7 RNA polymerase under control of the *lac*UV5 promoter. IPTG is required to induce expression of the T7 RNA polymerase.

The two strains carry a mutated *rne* gene (*rne131*) which encodes a truncated RNase E enzyme that lacks the ability to degrade mRNA, resulting in an increase in mRNA stability (see below).

The two strains are *E. coli* B/r strains and do not contain the *lon* protease. They are also deficient in the outer membrane protease, OmpT. The lack of these proteases reduces degradation of heterologous proteins expressed in the strains.

BL21 Star™(DE3)pLysS carries the pLysS plasmid which produces T7 lysozyme (see page 3). The BL21 Star™(DE3) strain does **not** carry a plasmid expressing T7 lysozyme.

rne131

The *rne* gene encodes the RNase E enzyme, an essential, 1061 amino acid *E. coli* endonuclease which is involved in rRNA maturation and mRNA degradation as a component of a protein complex known as a "degradosome" (Grunberg-Manago, 1999; Lopez *et al.*, 1999). Various studies have shown that the N-terminal portion of RNase E (approximately 584 amino acids) is required for rRNA processing and cell growth while the C-terminal portion of the enzyme (approximately 477 amino acids) is required for mRNA degradation (Kido *et al.*, 1996; Lopez *et al.*, 1999). The *rne131* mutation (present in the BL21 Star™ strains) encodes a truncated RNase E which lacks the C-terminal 477 amino acids of the enzyme required for mRNA degradation (Kido *et al.*, 1996; Lopez *et al.*, 1999). Thus, mRNAs expressed in the RNase E-defective BL21 Star™ strains exhibit increased stability. When heterologous genes are expressed in these strains from T7-based expression vectors, the yields of recombinant proteins generally increase.

Continued on next page

About the Kit, Continued

pLysS

The pLysS plasmid carried by the BL21 Star™(DE3)pLysS strain produces T7 lysozyme to reduce basal level expression of the gene of interest. pLysS confers resistance to chloramphenicol (Cam^R) and contains the p15A origin. This origin allows pLysS to be compatible with pUC- or pBR322-derived plasmids.

Expression of Heterologous Genes

The BL21 Star™(DE3) and BL21 Star™(DE3)pLysS strains are suitable for high-level recombinant protein expression. Due to the increase in stability of mRNAs, we have observed higher basal expression of heterologous genes in BL21 Star™ strains than in some BL21 strains; therefore, these strains may not be useful for expression of toxic genes. To choose an appropriate BL21 Star™ strain to use for expression of your gene of interest, see page 5.

Note: Basal expression levels of heterologous genes are generally higher in BL21 Star™(DE3) cells than in BL21 Star™(DE3)pLysS cells. However, the overall yield of recombinant protein is generally higher in BL21 Star™(DE3) cells than in BL21 Star™(DE3)pLysS cells..

The BL21 Star™ strains are useful to express heterologous genes from any T7-based expression vector. In general, we recommend using the BL21 Star™(DE3) strain to express heterologous genes from low-copy number, T7-based plasmids (*e.g.* pET vectors). We have observed toxicity when expressing some heterologous genes from high-copy number plasmids (*e.g.* Invitrogen's pCR®T7 vectors) in BL21 Star™(DE3) cells. These effects are alleviated when BL21 Star™(DE3)pLysS cells are used; therefore, we recommend using the BL21 Star™(DE3)pLysS strain to express heterologous genes from high-copy number, T7-based plasmids.

Continued on next page

General Handling

Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature and mechanical lysis caused by pipetting. Transformation should be started immediately after thawing the cells on ice. Mix the transformation reaction by swirling or tapping the tube gently, not by pipetting.

Important

BL21 Star™(DE3) and BL21 Star™(DE3)pLysS cells require IPTG to induce expression of the T7 RNA polymerase from the *lac*UV5 promoter. IPTG is available from Invitrogen (Catalog no. 15529-019). For more information, see our Web site or call Technical Support (see page 15).

Product Specifications

One Shot® BL21(DE3), BL21(DE3)pLysS, and BL21(DE3) pLysE cells are qualified based on the following criteria:

50 µL of competent cells are transformed with 10 pg of supercoiled pUC19 plasmid DNA. Transformed cultures are plated on LB plates containing 50 µg/mL ampicillin and the transformation efficiency is calculated. Test transformations are performed in triplicate. Transformation efficiency should be:

- $>1 \times 10^8$ cfu/µg DNA for BL21(DE3) cells
- $>1 \times 10^8$ cfu/µg DNA for BL21(DE3)pLysS cells
- $>1 \times 10^7$ cfu/µg DNA for BL21(DE3)pLysE cells

Untransformed cells are plated on:

- LB plates containing 50 µg/mL ampicillin to verify the absence of ampicillin resistant contamination.
- LB plates as a lawn to verify the absence of phage contamination.
- LB plates containing 34 µg/mL chloramphenicol for selection of pLysS or pLysE (for BL21(DE3)pLysS or BL21(DE3)pLysE, respectively)

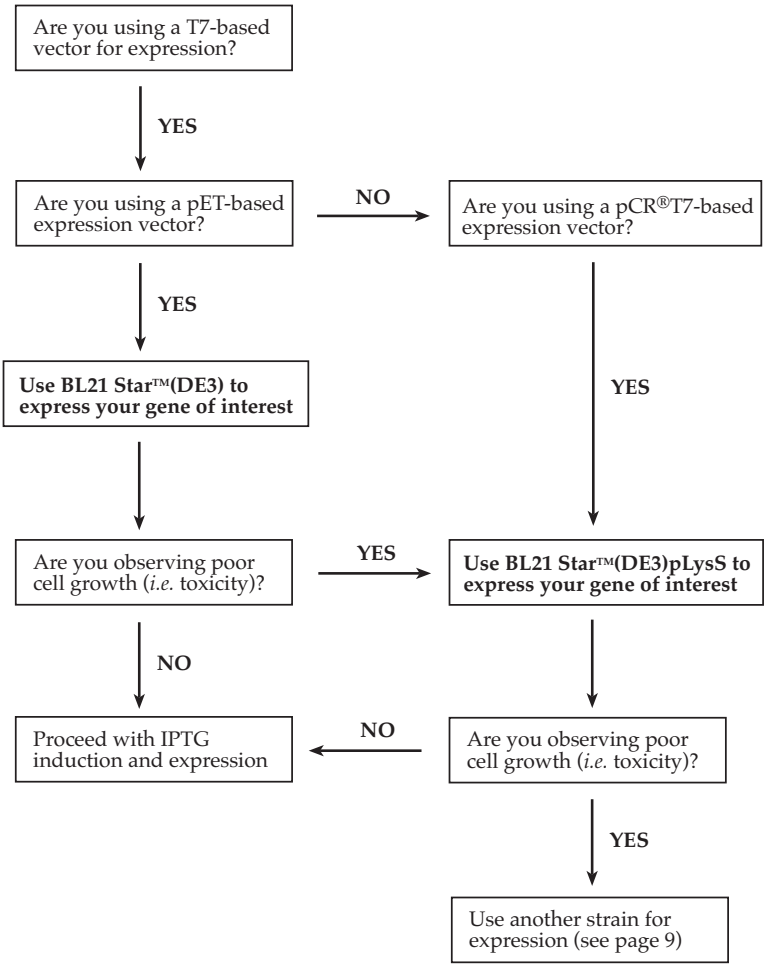
Intended Use

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

Choosing a BL21 Star™ Strain

Choosing a BL21 Star™ Strain for Expression

The flowchart below provides some general guidelines to help you choose the appropriate BL21 Star™ strain to use in your expression experiments.



For more information about indications of toxicity in bacterial cells, see the **Expression Guidelines**, page 9.

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Choosing a BL21 Star™ Strain, Continued

Comparison with Other BL21 Strains

We have compared expression of many heterologous genes from T7-based expression vectors in the RNase E-defective BL21 Star™ strains (BL21 Star™(DE3) or BL21 Star™(DE3)pLysS) to non-RNase E-defective BL21 strains (BL21(DE3) or BL21(DE3)pLysS). In most cases, the yield of recombinant protein obtained from the BL21 Star™ strain ranged from 2 to 10-fold greater than that from the corresponding non-RNase E-defective BL21 strain. The table below provides a representative list of recombinant proteins which we have found to be expressed to higher levels in the BL21 Star™ strains when compared to the non-RNase E-defective BL21 strains. Note that the pET-based vectors contain the *lacI* gene and the T7/*lac* promoter.

Recombinant Protein	Expression Vector	BL21 Star™ Strain	BL21 Strain
Firefly luciferase	pET-based	BL21 Star™(DE3)	BL21(DE3)
Vaccinia topoisomerase I	pET-based	BL21 Star™(DE3)	BL21(DE3)
E. coli β-galactosidase (*lacZ*)	pCR®T7-based	BL21 Star™(DE3)pLysS	BL21(DE3)pLysS
Human RARγ	pCR®T7-based	BL21 Star™(DE3)pLysS	BL21(DE3)pLysS
Human TFIIB (*GTF2B*)	pCR®T7-based	BL21 Star™(DE3)pLysS	BL21(DE3)pLysS
Human BDNF receptor (*NTRK2*)	pCR®T7-based	BL21 Star™(DE3)pLysS	BL21(DE3)pLysS
Human Cdk5 (*CDK5*)	pCR®T7-based	BL21 Star™(DE3)pLysS	BL21(DE3)pLysS

Note: Some heterologous genes may not be expressed to higher levels in the BL21 Star™ strains when compared to non-RNase E-defective BL21 strains.

Basic Transformation Procedure

Introduction

A basic transformation protocol for BL21 Star™(DE3) and BL21 Star™(DE3)pLysS cells is provided below. Once you have selected transformants, we recommend that you proceed directly to expression using your own protocol. **Note that BL21 Star™(DE3) and BL21 Star™(DE3)pLysS are designed to be used for expression, not cloning or subcloning.**

Materials Supplied by the User

- Plasmid DNA (ready for transformation)
- 42°C water bath
- 37°C shaking and non-shaking incubator
- Ice bucket with ice
- Spectrophotometer to measure optical density of the cell cultures
- Microcentrifuge tube rack (optional)

Before Starting

- Prepare LB agar plates containing the appropriate concentration of antibiotic (to select for your plasmid). If you are transforming DNA into BL21 Star™(DE3)pLysS cells, add 34 µg/mL chloramphenicol (to select for pLysS)
- Equilibrate a water bath to 42°C
- Warm the vial of SOC medium to room temperature
- Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation)

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Basic Transformation Procedure, Continued

Basic Transformation Procedure

1. Thaw one vial of One Shot® cells on ice per transformation.
2. Add 5–10 ng of DNA, in a volume of 1–5 µL to the cells and mix by tapping gently. **Do not mix cells by pipetting.**
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat shock the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. **Do not mix or shake.**
5. Remove the vial(s) from the 42°C bath and quickly place on ice.
6. Add **250 µL** of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; use proper sterile technique to avoid contamination.)
7. Secure the vial(s) in a microcentrifuge rack with tape. Place the rack in a shaking incubator, and shake the vial(s) at 37°C for 1 hour at 225 rpm.
8. Plate two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Include 34 µg/mL chloramphenicol if using BL21(DE3)pLysS or BL21(DE3)pLysE cells. Select two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. The remaining transformation reaction may be stored at 4°C and plated out the next day, if needed.
9. Invert the plates and incubate at 37°C overnight.
10. Select transformants from the plates and culture as described on page 9.

    Note: Clones may exhibit differences in expression of heterologous genes. We recommend choosing 3–4 transformants when characterizing clones for protein expression.

Expression Guidelines

Introduction

If you have an expression protocol for the plasmid that you are working with, we recommend that you use your own protocol. This section provides some general guidelines for the use of T7 RNA polymerase-based expression plasmids in BL21 Star™(DE3) or BL21 Star™(DE3)pLysS cells.

Transform your expression plasmid into a strain that does not bear the gene for T7 RNA polymerase (*i.e.* TOP10, DH5α™) and maintain your construct in this strain. Use BL21 Star™(DE3) or BL21 Star™(DE3)pLysS cells **for expression only**.

BL21 Star™ Strains

The BL21 Star™ strains are suitable for high-level recombinant protein expression. In general, the yield of recombinant protein obtained from BL21 Star™(DE3) cells is higher than the yield from BL21 Star™(DE3)pLysS cells.

Using BL21 Star™(DE3) pLysS

We recommend using the BL21 Star™(DE3)pLysS strain if:

- You are using a high-copy, T7-based vector to express your gene of interest
- You observe growth inhibitory effects (*i.e.* toxicity) when using BL21 Star™(DE3) (see page 12)
- You are expressing a known toxic gene

Continued on next page

Expression Guidelines, Continued

T7 RNA Polymerase and Toxic Genes

In the BL21 Star™(DE3) and BL21 Star™(DE3)pLysS strains, the T7 polymerase gene is controlled by the IPTG-inducible *lacUV5* promoter. Because of the extremely high activity of T7 RNA polymerase and the increased stability of mRNAs, some basal level expression of the gene of interest will likely occur in uninduced cells. This creates problems in cases where the gene of interest is toxic to bacterial cells. In these cases, expression of the toxic gene under uninduced conditions leads to selection of cells that express the lowest levels of the toxic gene. These cells are often unable to express high levels of the gene of interest upon IPTG induction of the T7 polymerase.

The BL21 Star™(DE3)pLysS strain produces T7 lysozyme which helps to reduce basal levels of T7 RNA polymerase. Although levels are reduced, the cells may still contain a small amount of T7 RNA polymerase.

Continued on next page

Expression Guidelines, Continued

Expression Guidelines

The following guidelines assume that expression of your gene is not toxic to *E. coli*. If you are working with a toxic gene, some additional precautions may be taken (see the next page).

- Following transformation (see page 8), pick 3–4 transformants for overnight culture in 5 mL LB medium containing antibiotic to select for your expression plasmid. If using BL21 Star™(DE3)pLysS, add 34 µg/mL chloramphenicol to select for pLysS. Grow overnight at 37°C with shaking until the OD_{600} reaches 0.6–1.0.
- Use the overnight cultures to inoculate fresh LB medium containing antibiotic to an OD_{600} of 0.05–0.1 (~1:20 dilution of the overnight culture). This dilution allows the cells to quickly return to logarithmic growth and reach the appropriate cell density. Use a volume appropriate for taking time points, if desired.

  Note: If you are using BL21 Star™(DE3)pLysS, you may choose not to include chloramphenicol in these cultures. Generally, the cells will not lose the pLysS plasmid during the limited number of cell doublings that occur in the growth and induction stages.
- Use the remainder of each overnight culture to create glycerol stocks. Once you have identified the clone that best expresses your protein, you can use the glycerol stock to perform additional expression experiments.
- Grow the cultures until they reach mid-log phase (OD_{600} ~0.4; 2 to 3 hours).
- Induce the cultures by adding IPTG to a final concentration of 0.5 mM and culture for an additional 2–3 hours. You may also take time points to analyze for optimal expression of your protein.
- Analyze clones by western blot or enzymatic assay to determine which clone best expresses your protein of interest. Use the glycerol stock created from this clone for expression experiments. If you find that expression levels in subsequent inductions decrease, or you find that you lose your plasmid, your protein may be toxic to *E. coli* (see page 12 for additional information).

Continued on next page

Expression Guidelines, Continued

Indications of Toxicity

When expressing recombinant proteins in BL21 Star™ strains, we generally assume that the recombinant protein is toxic to bacterial cells when any of the following occurs:

- No transformants are obtained after following Steps 1-9 of the **Basic Transformation Protocol** on page 8 **OR** a combination of large and small, irregular colonies appears on the plate
- The initial culture does not grow (see previous page)
- It takes longer than 5 hours after a 1:20 dilution of the initial culture for the fresh culture to reach an OD_{600}=0.4 (see previous page)
- The cells lyse after induction with IPTG (see previous page)

Precautions

Review the guidelines below when basal level expression of a gene of interest is toxic. These guidelines assume that the T7 expression plasmid has been correctly designed and created.

- Use the BL21 Star™(DE3)pLysS strain for expression experiments. The strain produces T7 lysozyme to inhibit the action of T7 RNA polymerase and reduce basal level expression of the gene of interest.
- Propagate and maintain your expression plasmid in a strain that does not contain T7 RNA polymerase (i.e. TOP10, DH5α™, etc.).
- Perform a fresh transformation of BL21 Star™(DE3) pLysS cells before each induction experiment.
- Minimize the amount of time that the cells bearing the gene of interest are cultured before IPTG induction.
- Following transformation of BL21 Star™(DE3)pLysS cells, grow cells in SOC medium for 1 hour and go directly to protein expression. Do not plate the transformation mixture to select for individual clones. See next page for details.

Continued on next page

Expression Guidelines, Continued

Transformation/ Expression Protocol for Toxic Genes

This alternative protocol may be used with BL21 Star™(DE3)pLysS cells. Other protocols are suitable, depending on your needs.

Transformation

1. Follow the basic transformation protocol on page 8 through Step 7.
2. After growing the transformation reaction in SOC for 1 hour (page 8, Step 7), add the **entire** transformation reaction (300 µL) to 50–200 mL of LB medium pre-warmed to 37°C containing the appropriate selective antibiotic for your expression plasmid, and 34 µg/mL chloramphenicol.

Induction

3. Incubate the vial(s) with shaking at 37°C until the cells reach mid-log phase (OD_{600} = 0.3). **Note**: Doubling times may vary (30 to 90 minutes) depending on the protein expressed.
4. Add IPTG to a final concentration of 0.5–1 mM and grow for 2–3 more hours. You make take time points, if desired.
5. Harvest cells by centrifugation and use immediately for analysis, or store the cell pellet at –80°C.

Other Alternatives

If you are using BL21 Star™(DE3)pLysS cells and observe significant toxicity, you may want to try using the BL21-AI™ strain (Catalog no. C6070-03) available from Invitrogen to express your recombinant protein of interest. The BL21-AI™ strain contains a chromosomal insertion of the gene encoding T7 RNA polymerase into the *ara*B locus of the *araBAD* operon, allowing expression of T7 RNA polymerase to be tightly regulated by L-arabinose. For more information about the BL21-AI™ strain, see our website (www.invitrogen.com) or call Technical Support (see page 15).

Testing Transformation Efficiency

Introduction

To test the transformation efficiency of the competent cells contained in the One Shot® kit, use the supercoiled pUC19 plasmid supplied with the kit as described below. An extra vial of cells is included for this purpose.

Before Starting

- Prepare LB agar plates containing 50 µg/mL ampicillin.
- Equilibrate a water bath to 42°C.
- Warm the vial of SOC medium to room temperature.
- Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation).

Transformation

Follow the transformation protocol on page 8 to transform pUC19 into BL21 Star™(DE3) or BL21 Star™(DE3)pLysS. Use the specific modifications below.

- Transform cells with 1 µL (10 pg) of pUC19
- Plate 50 µL each onto two LB plates containing 50 µg/mL ampicillin.
- Calculate the transformation efficiency as transformants per 1 µg of plasmid (see below). The cells should have an efficiency of 1 x 10^8 transformants/µg of supercoiled plasmid.

Calculation

Use the formula below to calculate transformation efficiency.

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µL transformed cells}}{\text{X µL plated}} = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information.
- Access to the Invitrogen Online Catalog.
- Additional product information and special offers.

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SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

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Technical Support, Continued

Limited Warranty

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Purchaser Notification

Introduction

Use of any BL21 Star™ *E. coli* strain is covered under a number of different licenses including those detailed below.

Information for European Customers

The BL21 Star™(DE3) and BL21 Star™(DE3)pLysS strains are genetically modified and carry the bacteriophage DE3 lysogen containing the T7 RNA polymerase gene. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Limited Use Label License No: 30 T7 Expression System

The composition and/or use of this product may be claimed in U.S. Patent No. 5,693,489 licensed to Life Technologies Corporation by Brookhaven Science Associates, LLC. The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy, and is the subject of patents and patent applications assigned to Brookhaven Science Associates, LLC (BSA,). By provisions of the Distribution License Agreement granted to Invitrogen covering said patents and patent applications, Invitrogen grants you a non-exclusive sub-license under patents assigned to BSA for the use of this technology, including the enclosed materials, based upon the following conditions: 1 – these materials are to be used for non-commercial research purposes only. A separate license under patents owned by BSA is required for any commercial use, including the use of these materials for research purposes or production purposes by any commercial entity. Information about commercial license may be obtained from The Office of Technology Transfer, Brookhaven National Laboratory, Bldg. 475D, P.O. Box 5000, Upton, New York 11973-5000. Phone (516) 344-7134. 2 - No materials that contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase, may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this sub-license and agrees to be bound by its terms. This limitation applies to strains BL21(DE3), BL21(DE3)pLysS and BL21(DE3)pLysE, CE6, BL21-SI Competent Cells and any derivatives that are made of them. You may refuse this sub-license by returning this product unused in which case Invitrogen accept return of the product with a full refund. By keeping or using this product, you agree to be bound by the terms of this license.

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Lopez, P. J., Marchand, I., Joyce, S. A., and Dreyfus, M. (1999). The C-terminal Half of RNase E, Which Organizes the *Escherichia coli* Degradosome, Participates in mRNA Degradation but not rRNA Processing *in vivo*. Mol. Microbiol. *33*, 188-199.

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