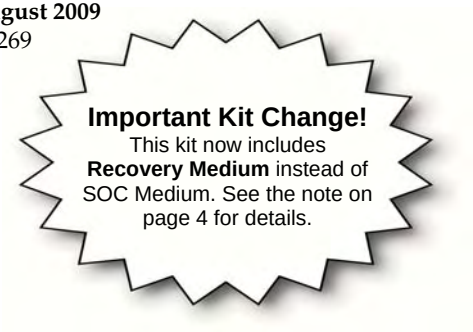
invitrogen™

One Shot® INV110 Competent Cells

Catalog no. C7171-03

Revision date: 20 August 2009
Manual part no. 25-0269

MAN0000103



User Manual

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Kit Contents and Storage

Shipping and Storage

One Shot® INV110 Competent Cells are shipped on dry ice. Upon receipt, remove the Recovery Medium and store at room temperature or at +4°C. Store the remainder of the kit at –80°C.

Kit Contents

Each kit contains the following:

- 21 tubes of chemically competent INV110 *E. coli* in 50 µl aliquots (transformation efficiency = 1×10^6 cfu/µg supercoiled DNA)
- Supercoiled pUC19 plasmid DNA (100 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8; 20 µl) for testing transformation efficiency
- Recovery Medium (10 ml) for plating (see **Important Note** below)

Important

Effective 20 August 2009, this kit now includes **Recovery Medium** instead of SOC Medium. Recovery Medium is a slightly richer formulation than SOC for cultivating recombinant strains of *E. coli*, and provides better lot-to-lot consistency. This change has been validated and does not affect the product's protocol or specifications.

Please continue to perform your transformation procedure as before, simply substituting Recovery Medium for SOC Medium. **All volumes, protocols, and handling procedures are the same for the new medium.**

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website at www.invitrogen.com/support. Search for the Certificate of Analysis by product lot number, which is printed on the box.

Information for European Customers

The INV110 *E. coli* strain is genetically modified and carries Tn*10* (Tet^R) to allow tetracycline selection of the host strain. 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.

Description of the Cells

Features of INV110

The INV110 *E. coli* strain contains the following features:

- *dam* and *dcm* deficiencies to allow restriction digestion with *dam*- and *dcm*-sensitive restriction enzymes (see below for more information)
- The *Δ(mcrC-mrr)* allele eliminates two restriction systems to allow more efficient transformation of DNA from highly methylated sources (i.e. eukaryotic DNA)
- The *end*A1 mutation to permit isolation of higher quality plasmid DNA
- The *lac*I^q allele for high expression of the Lac repressor
- Tn*10* to permit selection of the host strain using tetracycline

Genotype

F´ {*traΔ36 proAB lac*I^q *lacZΔM15*} *rps*L (Str^R) *thr leu end*A *thi*-1 *lacY galK galT ara ton*A *tsx dam dcm supE44 Δ(lac-proAB) Δ(mcr*C-*mrr)102*::Tn*10* (Tet^R)

dam and *dcm* Methylation

INV110 is a *dam* and *dcm* methylase-deficient *E. coli* strain. In *dam*$^+$ *dcm*$^+$ *E. coli* strains, the Dam and Dcm methylases methylate DNA at the following sites:

Methylase	Methylation Site
Dam	G^{m}ATC
Dcm	C^{m}C(A/T)GG

The *dam* and *dcm* deficiencies allow production of DNA that is unmethylated at these sites. Please refer to the catalog of your restriction enzyme supplier for information on methylation-sensitive restriction enzymes.

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The presence of the *dam* deficiency affects INV110 in a number of ways. Please be aware that INV110 cells have the following characteristics when compared to *dam*$^+$ *E. coli* strains:

- A higher mutation rate (greater than 250-fold)
- A higher recombination frequency and lower viability on plates
- A lower transformation efficiency

If you are isolating DNA from transformants, we recommend that you pick colonies and isolate DNA within one day after transformation. **INV110 should not be used to maintain your plasmid of interest for long-term storage.**

General Handling

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

Blue-White Screening

If blue-white screening is required to select for transformants, your LB agar plates must contain X-Gal and isopropyl -D-thiogalactoside (IPTG). INV110 One Shot® cells express the Lac repressor and require IPTG to induce expression from the *lac* promoter.

To perform blue-white screening, prepare the following stock solutions of X-Gal and IPTG:

- 40 mg/ml X-Gal in dimethylformamide (DMF)
- 100 mM IPTG in water (filter sterilize)

You may add X-Gal and IPTG to LB agar plates using one of the following methods:

1. Spread 40 µl of 40 mg/ml X-Gal and 40 µl of 100 mM IPTG on top of the agar. Let the X-Gal and IPTG diffuse into the agar for approximately 1 hour.
2. Add X-Gal and IPTG directly to the autoclaved agar prior to pouring your plates. When the autoclaved agar has cooled to 55°C, add X-Gal to a final concentration of 20 µg/ml and IPTG to a final concentration of 0.1 mM.

Transformation Protocol

Materials Supplied by the User

You will need the following items for transformation:

- 37°C shaking and non-shaking incubator
- 10 cm diameter LB agar plates containing 10 µg/ml tetracycline, the appropriate antibiotic for selection of your plasmid of interest (i.e. 50–100 µg/ml ampicillin), and X-Gal and IPTG, if desired
- Ice bucket with ice
- 42°C water bath
- A test tube rack to hold all transformation tubes so that they can all be put into a 42°C water bath simultaneously

Before Starting

- Equilibrate a water bath to 42°C
- Warm the vial of Recovery Medium to room temperature. ***Note:*** If you are using an older kit that contains SOC Medium, simply follow the protocol in this manual, substituting the SOC Medium for the Recovery Medium specified here.
- Spread X-Gal and IPTG onto LB agar plates containing tetracycline and the appropriate antibiotic, if desired
- Warm the plates in a 37°C incubator for 1 hour (use one plate for each transformation)

Transformation Procedure

Follow the instructions provided below to transform your plasmid of interest into INV110.

1. Centrifuge the vial(s) containing the plasmid DNA briefly and place on ice.
2. Thaw, on ice, one 50 µl vial of One Shot® cells for each transformation.
3. Pipet 1–5 µl (10–100 ng) of each DNA sample directly into the competent cells and mix by tapping gently. **Do not mix cells by pipetting.** The remaining DNA sample(s) can be stored at -20°C.
4. Incubate the vial(s) for 30 minutes on ice.

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Transformation Protocol, Continued

Transformation Procedure, continued

Procedure continued from previous page

5. Transfer all vials at the same time (i.e., in a rack) to the 42°C water bath and incubate for exactly 30 seconds. Do not mix or shake.
6. Remove vial(s) from the 42°C bath and place on ice.
7. Add **250 µl** of pre-warmed Recovery Medium to each vial. ***Important:*** Use sterile technique when handling the medium to avoid contamination.
8. Place the vial(s) in a microcentrifuge rack on its side and secure with tape to avoid loss of the vial(s). Shake the vial(s) at 37°C for 1 hour at 225 rpm in a shaking incubator.
9. Spread 20 µl to 200 µl from each transformation vial on a separate, labeled LB agar plate containing X-Gal and IPTG (if desired), 10 µg/ml tetracycline, and the appropriate antibiotic for selection of the plasmid of interest.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by restriction digest, PCR, or sequencing.

We recommend that you test the efficiency of the competent cells by using the supercoiled pUC19 plasmid supplied with the kit as described below.

- Prepare LB agar plates containing 10 µg/ml tetracycline and 50–100 µg/ml ampicillin.
- Transform 1 µl (100 pg) into 50 µl of competent cells according to the transformation procedure above.
- Incubate overnight at 37°C and count colonies. Calculate the transformation efficiency as transformants per 1 µg of plasmid using the formula below. The cells should have an efficiency of at least 1×10^6 transformants/µg of supercoiled plasmid.

Calculation

Use the formula below to calculate transformation efficiency.

$$\frac{\text{\# of colonies}}{\text{100 pg transformed DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µl total transformation volume}}{\text{X µl plated}} = \frac{\text{\# transformants}}{\text{µg plasmid DNA}}$$

Technical Support

World Wide Web

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

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

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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Technical Support, continued

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