

One Shot® INV110 Competent Cells

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Description

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)
- $\Delta(mcrC-mrr)$ allele to eliminate 2 restriction systems and allow more efficient transformation of DNA from highly methylated sources (i.e. eukaryotic DNA)
- *endA1* mutation to permit isolation of higher quality plasmid DNA
- *lacI^q* allele for high expression of the Lac repressor
- Tn10 to permit selection of the host strain using tetracycline

Contents

Each kit contains the following:

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

Important!

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 it provides better lot-to-lot consistency. This change has been validated and does not affect the product's protocol or specifications.

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.**

Genotype

F' {*tra* Δ 36 *proAB lacI^q lac* Δ M15} *rpsL* (Str^R) *thr leu endA thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ (lac-proAB) Δ (mcrC-mrr)102::Tn10 (Tet^R)*

Shipping and Storage

One Shot® INV110 Competent Cells are shipped on dry ice. Upon receipt, remove the Recovery Medium and store it at 15°C to 30°C or at 2°C to 8°C. Store the remainder of the kit at -85°C to -68°C.

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	GmATC
Dcm	CmC(A/T)GG

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

Caution!

The presence of the *dam* deficiency affects INV110 in a number of ways. 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 the DNA within one day after transformation. *Do not use INV110 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. Start transformation 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 μ L of 40 mg/mL X-Gal and 40 μ L 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 μ g/mL and IPTG to a final concentration of 0.1 mM.

Materials Supplied by the User

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

Before Beginning Transformation

- 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, 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. Store the remaining DNA sample(s) at –30°C to –10°C.
4. Incubate the vial(s) for 30 minutes on ice.
5. Transfer all vials at the same time (i.e., in a rack) to the 42°C water bath and incubate them for exactly 30 seconds. Do not mix or shake.
6. Remove the vial(s) from the 42°C bath and place them 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 them 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 20–200 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.

Testing Transformation Efficiency

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

1. Prepare LB agar plates containing 10 µg/mL tetracycline and 50–100 µg/mL ampicillin.
2. Transform 1 µL (100 pg) into 50 µL of competent cells according to the transformation procedure above.
3. Incubate overnight at 37°C and count the 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.



Calculating Transformation Efficiency

Use the formula below to calculate transformation efficiency:

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

Information for Non-U.S. Customers

The INV110 *E. coli* strain is genetically modified and carries Tn10 (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.

Safety Data Sheets (SDSs)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

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