One Shot® INVαF´Competent Cells

Catalog no. C2020-03 and C2020-06

Version J
13 April 2004
28-0123

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Overview

Introduction

The information in this manual covers the following kits:

Kit	Reactions	Catalog no.
One Shot® INVαF′ Chemically Competent *E. coli*	20	C2020-03
	40	C2020-06

Contents

- 21 or 41 tubes of competent INVαF′ *E. coli* in 50 µl aliquots (1 x 10^8 colonies/µg DNA)
- Supercoiled pUC19 plasmid (10 pg/µl in 5 mM Tris-HCl; 0.5 mM EDTA, pH8; 50 µl) for testing efficiency
- SOC medium (6 ml) for plating

Genotype

F′ *end*A1 *rec*A1 *hsd*R17 (r_k^-, m_k^+) *sup*E44 *thi*-1 *gyr*A96 *rel*A1 ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 λ^-

Materials Supplied by the User

- 42°C water bath
- 37°C incubator
- 10 cm diameter LB agar plates with appropriate antibiotic
- Ice bucket with ice

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. Swirl or tap the tube gently to mix reagents; do not mix by pipetting up and down. .

Important

INVαF′ One Shot® cells do not require IPTG to induce expression from the *lac* promoter.

If blue/white screening is required to select for transformants spread 40 µl of 40 mg/ml X-Gal in dimethylformamide. Let the X-Gal diffuse into the agar for approximately 1 hour.

Continued on next page

One Shot® Competent *E. coli*

All competent cells are qualified as follows:

- Cells are tested for transformation efficiency using the control plasmid included in the kit. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be ~1 x 10^9 cfu/µg DNA for chemically competent cells and >1 x 10^9 for electrocompetent cells.
- To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected.
- Untransformed cells are plated on LB plates 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

We recommend that you test the efficiency of the competent cells contained in the One Shot® kit. This can be accomplished by using the supercoiled pUC19 plasmid supplied with the kit as described below.

- Prepare LB agar plates containing 50 µg/ml ampicillin
- Transform 1 µl (10 pg) into 50 µl of competent cells according to the transformation protocol on the next page

Calculate the transformation efficiency as transformants per 1 µg of plasmid (see next page). The cells should have an efficiency of 10^8 transformants/µg of supercoiled plasmid.

Transformation

Before Starting

- Equilibrate a water bath to 42°C
- Warm the vial of SOC medium to room temperature
- Place an appropriate number of 10 cm diameter LB agar plates with antibiotic in a 37°C incubator to remove excess moisture (use one plate for each transformation). **Be sure to add X-Gal to the plates if necessary.**
- Obtain a test tube rack that will hold all transformation tubes so that they all can be put into a 42°C water bath at once.

Transformation Procedure

The instructions provided below are for general use. Specific instructions for particular applications such as TA Cloning® are provided in the respective manual. If you are using One Shot® kits in conjunction with Original TA Cloning®, refer to the manual for specific instructions.

1. Centrifuge the vial(s) containing the ligation reaction(s) briefly and place on ice.
2. Thaw on ice one 50 µl vial of One Shot® cells for each ligation/transformation.
3. Pipet 1 to 5 µl of each ligation reaction directly into the competent cells and mix by tapping gently. **Do not mix cells by pipetting.** The remaining ligation mixture(s) can be stored at -20°C.

   Use 1 µl of supercoiled pUC19 plasmid (10 pg/µl) for the transformation control.
4. Incubate the vial(s) on ice for 30 minutes.
5. Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
6. Remove vial(s) from the 42°C bath and quickly place them on ice.
7. Add **250 µl** of pre-warmed SOC medium to each vial. (SOC is a rich medium; good sterile technique must be practiced 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 exactly 1 hour at 225 rpm in a gyratory shaker-incubator.
9. Spread 50 µl to 100 µl from each transformation vial on separate, labeled LB agar plates. **Note**: Plate 50 µl from the transformation control.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies for further analysis by plasmid isolation, PCR, or sequencing.

Calculation

Use the formula below to calculate transformation efficiency.

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

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters:
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Continued on next page

Technical Service, Continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.