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A Geno Technology, Inc. (USA) brand name

Z-Competent™ *E. coli* Transformation

(Cat. # GZ-4, GZ-5)

INTRODUCTION

The Z-Competent *E. coli* Transformation™ is a new simple method for making competent *E. coli* cells for highly efficient E. coli transformation. The kit features a transformation efficiency of 2 x 10^8-1 x 10^9 transformants per µg supercoiled pUC19 plasmid DNA. The specific efficiency of the transformation varies according to the strain of E. coli used. The Cat.# GZ-4 is enough for 300 preps, while GZ-5 supplied with SOB Culture Medium is good for 100 preps.

ITEM(S) SUPPLIED

Description	**Cat. # GZ-4 300 preps**	**Cat. # GZ-5 100 preps**
Wash Buffer [2X]	15ml	6ml
Competent Buffer [2X]	15ml	6ml
Dilution Buffer	30ml	12ml
SOB Culture Medium	-	2 x 50ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components at 4°C. When stored and used properly, the kit is stable for one year.

PREPARATION BEFORE USE

The wash and competent buffers are supplied as 2X stock solutions. They need to be diluted to 1X by adding an equal volume dilution buffer.

- To Prepare 5ml 1X Wash Buffer: Mix 2.5ml Dilution Buffer and 2.5ml 2X Wash Buffer
- To Prepare 5ml 1X Competent Buffer: Mix 2.5ml Dilution Buffer and 2.5ml 2X Competent Buffer

Keep these freshly prepared 1X buffers ice cold. These buffers are stable for 2 days at 4-25°C.

PROTOCOL

The following protocol is based on a 50ml SOB *E. coli* culture. The volume may be adjusted according to your experimental needs.

Preparation of Competent Cells

Inoculate 0.5ml of fresh overnight *E. coli* culture grown in LB to 50ml SOB in a 500ml flask shaking vigorously at 20-25°C until the OD_{600} reaches 0.4-0.6. This usually takes around 10-36 hours depending on the *E. coli* strain used and the growing temperature.

NOTE: *Perform the following procedure on ice or at 4°C*

1. Place the culture in ice for 10 minutes and then pellet the cells at 2,500x g for 6 minutes at 4°C.
2. Remove the supernatant and resuspend the cells gently in 5ml ice-cold 1X Wash Buffer, then r-pellet as in step 1.
3. Remove the supernatant completely and resuspend the cells gently in 5ml ice-cold 1X Competent Buffer
4. Aliquot 100µl of the competent cells into sterile microfuge tubes on ice.
5. At this stage the cells are ready for transformation experiments or can be stored at -70°C for later use. Hereinafter, the competent cells are called “Z-competent”.

Transformation of Z-Competent Cells

1. Take a tube of frozen or freshly prepared Z-competent cells on ice.
2. Add 1-5µl DNA and gently mix
   NOTE: *Keep the DNA in a volume less than 5% of the volume of the competent cells.*
3. Incubate on ice for 15-60 minutes.
4. Spread 50-100µl on an appropriate plate pre-warmed to 37°C.
5. Incubate the plate at 37°C or appropriate temperature for the colonies to grow.

Tips for Quick Transformation

If your experiment does not require very high transformation efficiency (i.e. when using plasmid stock to transform *E. coli*), incubate the DNA and cells on ice for 2-5 minutes and spread directly on to pre-warmed plates.

Important Notes for High Efficiency Transformation

1. Incubation Time

For standard transformation an incubation time of 60 minutes is recommended.

2. Pre-warm Agar Plate

Cold plates dramatically decrease transformation efficiency. It is strongly recommended that agar plates be pre-warmed at 37°C or at least 20°C.

3. Addition of SOC

Addition of SOC can increase the transformation efficiency by 2-3 folds. After incubating on ice, add 4 volumes of SOC (400µl of SOC to 100µl of transformation mixture) and incubate for one hour at 37°C. Spread the mixture directly onto pre-warmed plates. In most situations, this step is not needed. Reducing agents, such as DTT and 2-ME, are not needed for this procedure.

4. Culture Conditions

Cells harvested at lower density (OD_{600} 0.2-0.6) are usually more competent than cells harvested at high density (OD_{600} >0.6). The *E. coli* cells are more competent when the culture is grown at 20-25º C. Higher temperatures such as 30°C or 37°C decrease the transformation efficiency by 2-10 fold. Cells can also be harvested at lower density (OD_{600} 0.2-0.4) and re-suspended at a smaller volume such as 1-3ml instead of 5ml as recommended in the standard protocol.

APPENDIX:

Preparation of SOB Culture Medium (1L volume):

Mix the following ingredients:

- *20g Bactro-tryptone*
- *5g Yeast extract*
- *0.58g NaCl*
- *0.17g KCl*
- *10ml 1M $MgCl_2$*
- *10ml 1M $MgSO_4$*

Adjust pH to 6.0-7.0 and autoclave.

Open the bottle under the laminar flow hood and use aseptic techniques.

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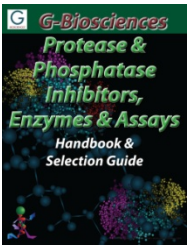

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