ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Premade Z-Competent™ *E. coli* Cells

Highlights

- Feature fast transformation kinetics: No heat shock, no lengthy incubations, no outgrowth procedures, no wait!
- High transformation efficiencies: 10^8-10^9 transformants/µg plasmid DNA.
- Simple: Mix DNA with cells for a few seconds and plate. *Mix & Go!*

Contents

Product Contents and Description ……..……..1
Protocol……...2, 3
Appendix...……..3
Ordering Information...……...4
List of Related Products...……...……5

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

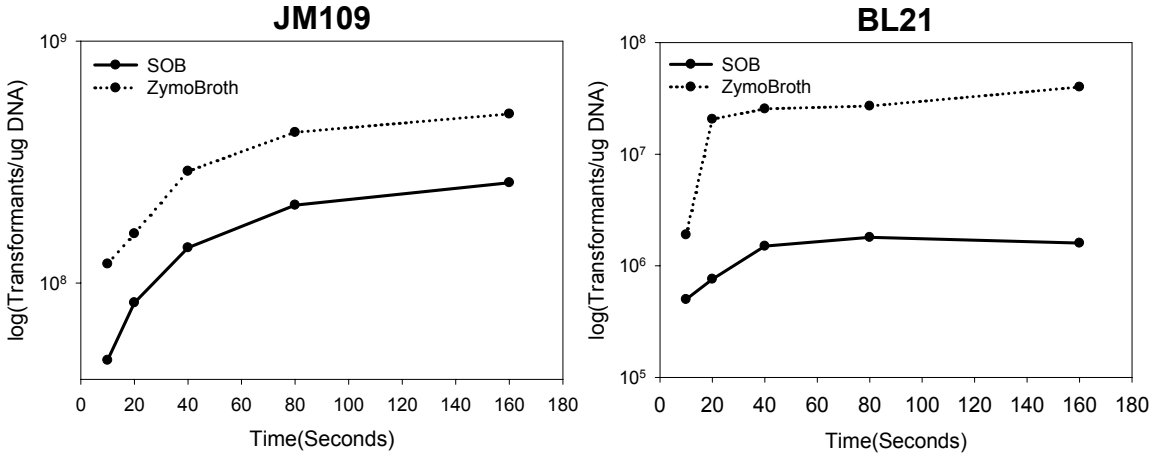
Product Contents

	Format		Storage Temp.
Z-Competent™ *E. coli* Cells	10x100 µl Single-Tube Aliquots	96-Well Format (12x8-Well Strips)	< -70°C
Instruction Manual	1	1	-

Note – Chemically Z-Competent™ cells are stable for 6 months at < -70°C. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Product Description

Z-Competent™ *E. coli* are premade chemically competent cells used for simple and highly efficient DNA transformation. Z-Competent™ *E. coli* cells are made chemically competent by a unique method using **ZymoBroth™** that completely eliminates the need for heat shock and related procedures. For transformation, DNA can be added directly to Z-Competent™ cells and the mixture spread directly to a culture plate - *Mix & Go!* Transformation efficiencies typically range from 10^8–10^9 transformants/µg of pUC19 DNA (see figures below), which make the cells optimal for cloning, sub-cloning, library construction, etc. Premade Z-Competent™ cells are supplied as a pack of 10 convenient 100 µl/tube aliquots or in a 96-well format (12x8-well strips) of 50 µl/tube.



Z-Competent™ *E. coli* cells prepared with ZymoBroth™ display fast transformation kinetics and high transformation efficiencies. Figures above show the transformation kinetics for Z-Competent™ JM109 and BL21 strains of *E. coli* generated using ZymoBroth™ and SOB growth media. Plasmid DNA (pUC19) was used for transformation and the data are the averages of three individual experiments.

Note -™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Protocol

Pre-warm culture plates to 37°C before starting.

For **Technical Assistance**, please contact **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Standard Transformation Prodedure

Single Tube Aliquots

1. Add 1-5 µl plasmid DNA to a tube of thawed Z-Competent™ cells on ice, mix gently for a few seconds (try to keep the added volume of DNA less than 5% of the total).
2. Incubate on ice for 2-5 minutes (maximum 60 minutes).
3. Spread 50-100 µl onto a pre-warmed culture plate. Incubate the plate at the appropriate temperature (e.g., 37°C) for the colonies to grow.

96-Well Format (8-Well Strips)

Thaw tube strips of frozen Z-Competent™ cells on ice (or 0-2°C).

1. Add 1-3 µl DNA to each tube of thawed Z-Competent™ cells and mix gently by tapping. Avoid repeated pipetting.
2. Incubate on ice (or 0-2°C) for 2-5 minutes (maximum 60 minutes).
3. Spread 25-50 µl of the mixtures onto pre-warmed culture plates.
4. Incubate the plate at 37°C or the appropriate temperature for the colonies to grow.

Note: The procedures above are for plasmids containing Ampicillin resistant markers. If Kanamycin, Tetracycline, Chloramphenicol, Erythromycin or any non-lactamase selection markers are used, an outgrowth step is required prior to plating. (see Notes Section 4 on page 3 regarding **Outgrowth**)

Important! Since chemically competent cells are extremely sensitive to changes in temperature, transformation should be performed immediately after thawing. The cells should avoid being exposed to room temperature for more than a few seconds. To mix cells after DNA addition, gently tap the tube with your fingers and then shake the tube downwards in a single motion from the elbow to collect the mixture at the bottom of the tube. Avoid repeated pipetting. Immediately transfer the transformation mixture(s) to ice or an ice bath (0°C).

Fast Transformation of Z-Competent™ Cells* - *Mix & Go!*

1. Add 1-5 µl plasmid DNA to a tube of thawed Z-Competent™ cells on ice, mix gently for a few seconds (try to keep the added volume of DNA less than 5% of the total).
2. Spread 50-100 µl of the mixture onto a pre-warmed (37°C) culture plate containing Ampicillin. Incubate the plate at the appropriate temperature (e.g., 37°C) for the colonies to grow.

*For Ampicillin selection only. For selection with other antibiotics, see Notes Section 4 on page 3.

Notes for High Efficiency Transformation

1. ***E. coli* Strains**
   Different *E. coli* strains vary in their ability to be transformed with DNA. Strains like JM109, C600, TG1, and DH5α* typically yield the highest transformation efficiencies.

2. **Incubation Time**
   The "*Mix & Go!*" procedure (page 2) will work for most transformations using Ampicillin selection and not requiring outgrowth (see Section 4 below). The highest transformation efficiencies can be obtained by incubating Z-Competent™ cells with DNA on ice for 2-5 minutes (60 minutes maximum) prior to plating.

3. **Prewarming Culture Plates**
   Chilled plates will decrease Z-Competent™ cell transformation efficiency. It is recommended that culture plates be pre-warmed to >20°C (preferably 37°C) prior to plating.

4. **Addition of SOC Medium to Transformation Mixtures (Outgrowth)**
   When selecting with Kanamycin, Tetracycline, etc., an outgrowth performed in SOC medium is required for efficient transformation. In most cases, this step can be omitted when selecting with Ampicillin. After the transformation mixture has incubated on ice for 5-10 min, add 4 volumes of SOC (400 µl of SOC to 100 µl of transformation mixture) and incubate for 1 hour at 37°C with gentle shaking at 200-300 rpm. Afterwards, spread the mixture directly onto pre-warmed culture plates. Reducing agents [e.g., DTT (Dithiothreitol) and 2-ME (β-mercaptoethanol)] are not required in this procedure.

Appendix

SOB Recipe: (1 Liter)

Mix the following ingredients:

20 g Bacto-Tryptone	0.58 g NaCl (or 2 ml of 5M NaCl)	10 ml 1M $MgCl_2$
5 g Yeast extract	0.19 g KCl (or 0.5 ml 1M KCl)	10 ml 1M $MgSO_4$

Add ddH_2O to a total volume of 1 liter.
Adjust pH to 6.0-7.0 with NaOH. Autoclave at 10 psi for 15-20 minutes.

SOC Recipe: (100 ml)

Add 1 ml of a 2 M filter-sterilized glucose solution or 2 ml of 20% (w/v) glucose solution to 100 ml of SOB medium.

LB Agar (1 Liter)

10 g of NaCl	10 g of Tryptone
15 g of Agar	5 g of Yeast Extract

Adjust the pH to 7.0 with 5 N NaOH. Autoclave at 15 psi for 15-20 minutes.

*DH5α is a trademark of Life Technologies.

Genotypes

JM109
F`traD36 lacI^q Δ(lacZ)M15 pro A+ B+ / e14$^-$ (McrA$^-$) Δ(lac-proAB)thi gyrA96 (Nal^r)endA1 hsdR17(r_k^- m_k^+)relA1 supE44 recA1 (1)
Comments: Partly restriction-deficient; good strain for cloning repetitive DNA(recA$^-$). Suppresses many amber mutations when glutamine is available but not the S_{100} or S_7 mutation of λ, e.g., λgt11. Can be used for M13 cloning/sequencing and blue/white screening.

XJa
recA1 supE44 endA1 hsdR17 (r_k^-,m_k^+) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB:: ΔR, cat F'[traD36 proAB+ lacI^q lacZ ΔM15]
Comments: Includes chromosomally encoded bacteriophage lambda R gene. Partly restriction-deficient; good strain for cloning repetitive DNA(recA$^-$). Suppresses many amber mutations when glutamine is acceptable but not the S_{100} or S_7 mutation of λ, e.g., λgt11. Can be used for M13 cloning/sequencing and blue/white screening.

XJa(DE3)
recA1 supE44 endA1 hsdR17 (r_k^-,m_k^+) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB:: ΔR, cat F'[traD36 proAB+ lacI^q lacZΔM15] ΔDE3
Comments: Includes chromosomally encoded bacteriophage lambda R gene and lambda DE DNA to express T7 RNA Polymerase. Partly restriction-deficient; good strain for cloning repetitive DNA(recA$^-$). Suppresses many amber mutations when glutamine is available but not the S_{100} or S_7 mutation of λ, e.g., λgt11. Can be used for M13 cloning/sequencing and blue/white screening.

C600
F$^-$ [e14-(McrA$^-$) or e14$^+$ (mcrA$^+$)] thr- 1leuB6 thi- 1 lacY1 supE44 rfbD1 fhuA21; the original C600 is EcoK r$^+$m$^+$ McrBC$^+$ (2,3)

DH5α
F$^-$ φ80dlacZΔM15 Δ(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(r_k^- m_k^+ phoA supE44 λ$^-$ thi-1 gyrA96 relA1
Comments: Insert stability due to Reca1 mutation. Can be used for blue/white screening, accepts large plasmids due to deoR mutation. High plasmid yield due to endA1 mutation.

HB101
F-Δ(gpt-proA)62 leuB6 supE44 ara-14 galkK2 lacY1 Δ(mcrC-mrr) rpsL20 (Str^r) xyl-5 mtl-1 recA13 (4)

TG1
F'traD36 lacI^q Δ(lacZ) M15 proA$^+$B$^+$ /supE Δ(hsdM-mcrB)5 (r_k^- m_k^- McrB$^-$) thi Δ(lac-proAB)

Ordering Information

Strain	Description	Cat. No.	Size*
JM109	For general cloning, blue-white selection, plasmid isolation. Healthy strain w/ transformation efficiency > 10^8.	T3003	10 x 100 µl
		T3005	96 x 50 µl
DH5α	For general cloning, blue-white selection, plasmid isolation. Slow growth w/ certain plasmids not stable. Transformation efficiency > 10^8.	T3007	10 x 100 µl
		T3009	96 x 50 µl
HB101	For general cloning, plasmid isolation. Transformation efficiency > 10^8.	T3011	10 x 100 µl
		T3013	96 x 50 µl
C600	For general cloning, plasmid isolation. Transformation efficiency > 10^8.	T3015	10 x 100 µl
TG1	For general cloning, blue-white selection, plasmid isolation. Transformation efficiency > 10^8.	T3017	10 x 100 µl
XJa Autolysis	JM109 w/ chromosomally inserted λ lysozyme gene that is inducible by arabinose. Z-Competent™ version is chemically competent with high transformation efficiency.	T3021	10 x 100 µl
XJa(DE3) Autolysis	JM109(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysogen encodes chromosomally-encoded T7 polymerase and is a suitable host for T7 driven expression of recombinant proteins (such as with the pET system). Z-Competent™ version are chemically competent with high transformation efficiency.	T3031	10 x 100 µl

* Available as (10) 100 µl single tube aliquots or (12) 50 µl 8-tube strips.

References:

1.) Sheridan, P. *et al*. **Phylogenetic Analysis of Anaerobic Psychrophilic Enrichment Cultures Obtained from a Greenland Glacier Ice Core**, Appl. Envir. Microbiol., Apr 2003; 69: 2153 – 2160.

2.) Yokobayashi, Y. *et al*. **From the Cover: Directed evolution of a genetic circuit**, PNAS, Dec 2002; 99: 16587 – 16591.

3.) Trent, J. *et al*. **A Ubiquitously Expressed Human Hexacoordinate Hemoglobin**, J. Biol. Chem., May 2002; 277: 19538 – 19545.

4.) Mourez, M. *et al*. **Mapping dominant-negative mutations of anthrax protective antigen by scanning mutagenesis**, PNAS, Nov 2003; 100: 13803 – 13808.

Popular E. coli Related Products from Zymo Research

Product	Description	Kit Size	Cat No.
Z-Competent™ *E. coli* Transformation Kit	Includes all buffers for making up to 20 ml Z-Competent™ *E. coli*. ZymoBroth™ medium is included.	20 ml	**T3001**
Z-Competent™ *E. coli* Transformation Buffer Set	Includes all buffers for making up to 60 ml Z-Competent™ *E. coli*. ZymoBroth™ medium is not included.	60 ml	**T3002**
ZymoBroth™	A specially formulated *E. coli* growth medium used in the preparation of highly competent *E. coli* for DNA transformation purposes. Can increase the transformation efficiency from 5 to 100-fold (depending on the *E. coli* strain).	100 ml 500 ml	**M3015-100** **M3015-500**
Ampicillin Trihydrate	Premade Ampicillin solution. Ampicillin inhibits bacterial cell wall synthesis. Commonly used to select for Ampicillin-resistant plasmid bearing strains of bacteria. Effective against both gram (-) and gram (+) bacteria.	5 x 1 ml 25 x 1 ml	**A1001-5** **A1001-25**
Chloramphenicol	Premade Chloramphenicol solution. Chloramphenicol inhibits bacterial protein synthesis by binding 50S ribosomal subunit. Commonly used for the amplification of vectors in gram (-) bacteria. Effective against both gram (-) and gram (+) bacteria and some mycobacteria.	5 x 1 ml 25 x 1 ml	**A1002-5** **A1002-25**
Kanamycin Sulfate	Premade Kanamycin solution. Kanamycin inhibits bacterial protein synthesis by binding 70S ribosomes resulting in dysfunctional translation of mRNA commonly used to select for cosmid vectors. Effective against both gram (-) and gram (+) bacteria.	5 x 1 ml 25 x 1 ml	**A1003-5** **A1003-25**
Tetracycline Hydrochloride USP	Premade Tetracycline solution. Tetracycline inhibits bacterial protein synthesis by binding the 30S ribosomal subunit. Effective against both gram (-) and gram (+) bacteria.	5 x 1 ml 25 x 1 ml	**A1004-5** **A1004-25**
Rattler™ Plating Beads	Sterile 5 mm plating beads are convenient and easy to use. No flaming required. Spread cells evenly over the entire culture plate surface.	1 Bottle 5 Bottles	**S1001** **S1001-5**

**Bulk quantities are available upon request. Please contact: busdev@zymoresearch.com or call 1-888-882-9682 for assistance.*