DNA Purification Made Simple

Mix & Go Competent *E. coli* Cells

Premade chemically competent cells used for simple and highly efficient DNA transformation.

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*

Catalog Numbers:
T3007, T3009, T3010, T3011, T3013, T3003, T3005, T3017, T3019, T3020

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

	Format			Storage
***Mix & Go* Competent *E. coli* Cells**	10 x 100 µl Single – Tube Aliquots	96 x 50 µl Aliquots (12 x 8-Tube Strips)	96 x 50 µl Aliquots (96-well PCR plates)	< -70°C
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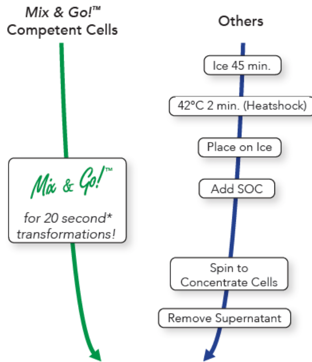
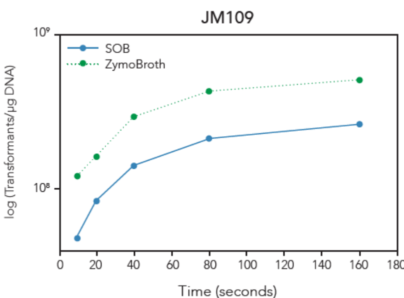
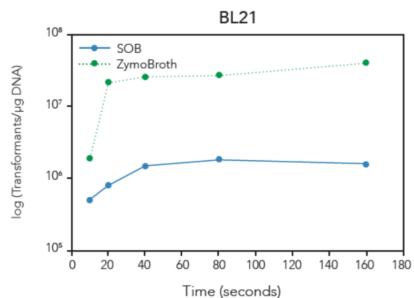
Product Description

Mix & Go E. coli are premade chemically competent cells used for simple and highly efficient DNA transformation. *Mix & Go E. coli* cells are made chemically competent by a unique method that completely eliminates the need for heat shock and related procedures. For transformation, DNA can be added directly to *Mix & Go* cells and the mixture spread directly to a culture plate. 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 *Mix & Go* cells are supplied as a pack of 10 convenient 100 µl/tube aliquots or in a 96-well format (12 x 8 - tube strips) of 50 µl/tube.



*Ampicillin selection only





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

Protocol

Pre-warm culture plates to 37°C before starting. Since chemically competent cells are extremely sensitive to changes in temperature, transformation should be performed immediately after thawing.

Transformation Procedure

Single Tube Aliquots

1. To a tube of *Mix & Go* cells thawed on ice, add 1-5 µl plasmid DNA[1], and then mix[2] gently for a few seconds. (For *Mix & Go* Transformation, go to Step 3 directly.)

2. Immediately place on ice and incubate for 2-5 minutes (maximum 60 minutes).

3. Spread 50-100 µl onto a pre-warmed culture plate (Ampicillin selection only, see note below). Incubate the plate at the appropriate temperature (*e.g.*, 37°C) for the colonies to grow.

96- Well Format (8-Tube Strips or PCR Plates)

1. To each tube (or well) of *Mix & Go* cells thawed on ice, add 1-3 µl plasmid DNA[1], and then mix[2] gently for a few seconds. (For *Mix & Go* Transformation, go to Step 3 directly.)

2. Immediately place on ice and incubate for 2-5 minutes (maximum 60 minutes).

3. Spread 25-50 µl of the mixtures onto pre-warmed culture plates (Ampicillin selection only, see note below). Incubate the plate at the appropriate temperature (*e.g.*, 37°C) 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 Page 4, Notes Section 2 and 4 regarding **Incubation Time & Outgrowth**).

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1 Keep the added volume of DNA less than 5% of the total.

2 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 exposing the cells to room temperature for more than a few seconds at a time.

Appendix

Notes for High Efficiency Transformation

1. ***E. coli* Strains**
   Different *E. coli* strains vary in their ability to be transformed with DNA. Strains like DH5 Alpha, JM109, and TG1 typically yield the highest transformation efficiencies.

2. **Incubation Time**
   The "*Mix & Go*" procedure (page 3) 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 *Mix & Go* cells with DNA on ice for 2-5 minutes (60 minutes maximum) prior to plating.

3. **Prewarming Culture Plates**
   Chilled plates will decrease *Mix & Go* 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.

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 S100 or S7 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 S100 or S7 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 S100 or S7 mutation of λ, e.g., λgt11. Can be used for M13 cloning/sequencing and blue/white screening.

DH5 Alpha
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.

Zymo 10B (Same as DH10B)
F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG λ-

Comments: Can be used for blue/white screening and is ideal for cDNA generation and library construction.

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)

Media Recipes

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.

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.

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 (12 x 8-Tube Strips)
DH5 Alpha	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 (12 x 8-Tube Strips)
		T3010	96 x 50 µl (PCR Plates)
HB101	For general cloning, plasmid isolation. Transformation efficiency > 10^8.	T3011	10 x 100 µl
		T3013	96 x 50 µl (12 x 8-Tube Strips)
TG1	For general cloning, blue-white selection, plasmid isolation. Transformation efficiency > 10^8.	T3017	10 x 100 µl
Zymo 10B (Same as DH10B)	For general cloning, blue-white selection, plasmid isolation. Transformation efficiency > 10^8. Ideal for cDNA generation and library construction.	T3019	10 x 100 µl
		T3020	96 x 50 µl (12 x 8-Tube Strips)
XJa Autolysis™	JM109 w/ chromosomally inserted λ lysozyme gene that is inducible by arabinose.	T3021	10 x 100 µl
XJb (DE3) Autolysis™	BL21(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysogen encodes chromosomally encoded T7 polymerase and is therefore a suitable host for expression of recombinant proteins under the control of the T7 promoter, such as in the pET system.	T3051	10 x 100 µl

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