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ZYMO RESEARCH

DNA Purification Made Simple

Mix & Go E. coli Transformation Kit & Buffer Set

For generating Mix & Go chemically competent cells from most *E. coli* lab strains for rapid, reliable, and highly efficient DNA transformation.

Highlights

- Simple method for generating *Mix & Go* chemically competent *E. coli* for rapid, reliable, and highly efficient DNA transformation (>10^8 transformants/µg plasmid DNA).
- Straightforward procedure: grow, wash, and then resuspend cells.
- The *Mix & Go E. coli* Transformation Kit (T3001) features a specially formulated **ZymoBroth™** growth medium that dramatically increases *E. coli* transformation efficiency, typically on the order of 5 to 100-fold for most lab strains.
- *Mix & Go* cells can be transformed in seconds without heat shocking, lengthy incubations, or outgrowth steps.

Catalog Numbers:
T3001, T3002

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

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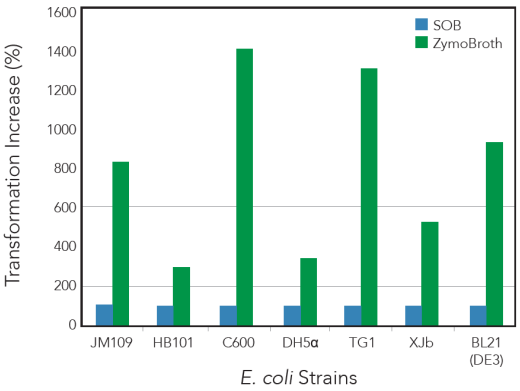
Product Contents

	Mix & Go E. coli Transformation Kit[1] **T3001** (20 ml)	*Mix & Go E. coli* Transformation Buffer Set[2] **T3002** (60 ml)	Storage
ZymoBroth™	200 ml	(Not Included)	Room Temp.
Wash Buffer (2X)	10 ml	30 ml	0-8°C
Competent Buffer (2X)	10 ml	30 ml	0-8°C
Dilution Buffer	20 ml	60 ml	0-8°C
Instruction Manual	1	1	-

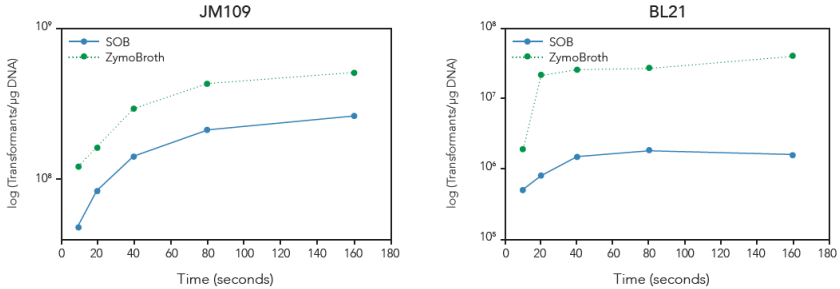
1 Includes all buffers for making up to 20 ml *Mix & Go E. coli*. **ZymoBroth™** growth medium is included.
2 Includes all buffers for making up to 60 ml *Mix & Go E. coli*. **ZymoBroth™** growth medium is **not** included.

Product Description

The ***Mix & Go E. coli* Transformation Kit & Buffer Set** are simple methods for generating *Mix & Go* chemically competent *E. coli* for rapid, reliable, and highly efficient DNA transformation. The methods eliminate the requirement of heat-shocking and related procedures. Instead, transformation can be performed by adding DNA to prepared *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 but can vary depending on the strain of *E. coli*. Most *E. coli* strains respond well to the Mix & Go preparation method and demonstrate fast transformation kinetics (see figures below).



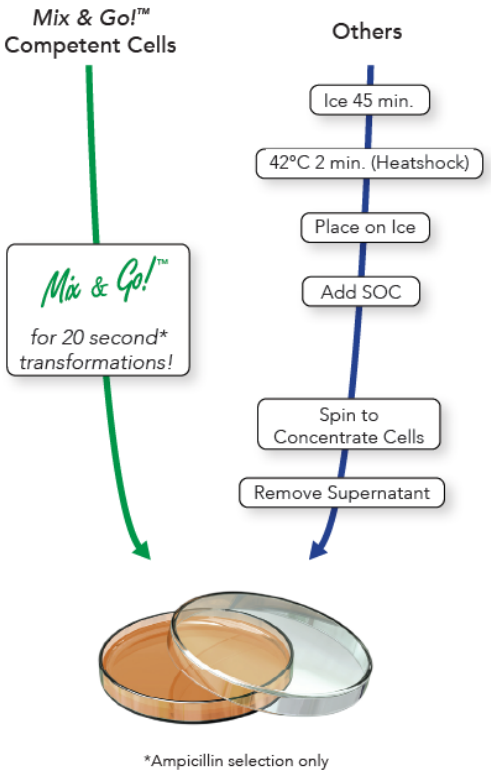
Transformation efficiencies of strains generated with ZymoBroth™ and SOB media. ZymoBroth™ dramatically increases the transformation efficiencies of a broad range of *E. coli* strains. Generally, ZymoBroth™ enhances transformation efficiencies better for difficult-to-transform strains.



Transformation kinetics. *Mix & Go!*™ *E. coli* prepared with ZymoBroth™ display fast transformation kinetics and high transformation efficiencies. pUC19 DNA was used for transformation and the data are the averages of three individual experiments.

The procedures are easy. Simply culture the *E. coli* strain of your choice in **ZymoBroth™** medium (or SOB), wash and then resuspend the cells in the provided uniquely formulated buffers. The cells are now ready for transformation!

The ***Mix & Go E. coli* Transformation Kit** (T3001) includes all buffers for making up to 20 ml *Mix & Go E. coli* from your favorite lab strains. **ZymoBroth™** growth medium is included. The ***Mix & Go E. coli* Transformation Buffer Set** (T3002) includes all buffers for making up to 60 ml *Mix & Go E. coli* from your favorite lab strains, but **ZymoBroth™** growth medium is not included.



Protocol

The following procedure is for a 50 ml *E. coli* culture in **ZymoBroth™** (supplied with T3001 only) or SOB medium (see appendix for recipe); however, the volume can be adjusted according to your specific requirements.

Preparation of *Mix & Go* Cells

1. Use 0.5 ml of fresh, overnight *E. coli* culture grown in LB to inoculate 50 ml **ZymoBroth™** or SOB medium in a 500 ml culture flask. Shake culture vigorously (150 - 250 rpm) at the appropriate temperature* until the OD_{600nm} is 0.4 - 0.6.

   Buffer Preparation Prior to Harvesting the Cells…

   - ✓ The **Wash** and **Competent Buffers** are provided as 2X stock solutions. They need to be diluted to 1X by adding an equal amount of **Dilution Buffer**.
   - ✓ To prepare 5 ml of 1X **Wash Buffer**: Add 2.5 ml **Dilution Buffer** and 2.5 ml of 2X Stock **Wash Buffer.**
   - ✓ To prepare 5 ml of 1X **Competent Buffer**: Add 2.5 ml **Dilution Buffer** and 2.5 ml of 2X Stock **Competent Buffer**.
   - ✓ Please keep these freshly prepared 1X Buffers ice cold. These 1X Buffers are good for 2 days at 0-25°C.

   <u>Important</u>! Each step of the following procedure should be done on ice or at 0-4°C.

2. Transfer the culture from Step 1 to ice. After 10 minutes, pellet the cells by centrifugation at 3,000 - 3,700 rpm (i.e., 1,600 - 2,500 x *g*) for 10 minutes at 0-4°C.

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* A study by Inou (Gene, 96:23-28, 1990) has shown *E. coli* cells to be highly competent when grown at 18-26°C prior to their preparation. In most cases, this can be achieved by shaking at room temperature for about 10-36 hours. This procedure is covered by US Patent No. 4,981,797 issued to Life Technologies, Inc. No license to use this technology is conveyed expressly or by implication to the purchaser by purchase of the *Mix & Go E. coli* Transformation Kit or *Mix & Go E. coli* Transformation Buffer Set

3. Remove the supernatant and resuspend the cells gently in 5 ml ice-cold 1X **Wash Buffer**. Re-pellet the cells as in Step 2.

4. Completely remove the supernatant and gently resuspend the cells in 5 ml ice-cold 1X **Competent Buffer**.

5. Aliquot (on ice) 0.1-0.2 ml of the cell suspension into sterile microcentrifuge tubes. Cells are now ready for transformation with DNA or can be stored below -70°C for transformation at a later time.

   Note: The prepared competent cells are referred to as "*Mix & Go*" in the procedures that follow.

Fast Transformation of *Mix & Go* Competent Cells*

1. Add 1-5 µl plasmid DNA to a tube of thawed *Mix & Go* 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 next page.

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 JM109, C600, TG1, DH5α, XL10 Gold, and BL21 and its derivatives typically yield the best results when prepared with the *Mix & Go E. coli* Transformation Kit.

2. **Incubation Time**
   The "*Mix & Go*" procedure can be used 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 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 for this procedure.

5. **Culture Conditions**
   E. coli cells become highly competent when cultured at 20-33°C prior to preparation. Higher temperatures (*i.e.*, 33°C-37°C) can decrease the transformation efficiency 2 to 10-fold. Also, cells can be harvested at lower densities (e.g., OD_{600nm} 0.2-0.4) and resuspended in smaller volumes (*e.g.*, 1-3 ml vs. 5 ml as recommended in the standard procedure). Cells harvested at lower densities (OD_{600nm} 0.2-0.6) are usually "more competent" than those cells harvested at higher densities (OD_{600nm} >0.6).

Media Recipes

Although SOB has traditionally been used for *Mix & Go* cell preparation, **ZymoBroth™** is now the medium of choice for the generation of *Mix & Go E. coli* that exhibit fast and highly efficient transformation kinetics. However, SOB can still be used in both ***Mix & Go E. coli* Transformation Kit and Buffer Set** procedures.

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 MgCl2
- ✓ 5 g Yeast extract
- ✓ 0.19 g KCl (or 0.5 ml 1M KCl)
- ✓ 10 ml 1M MgSO4

Add ddH2O 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.

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

Product Description	Catalog No.	Size
***Mix & Go E. coli* Transformation Kit** Includes all buffers for making up to 20 ml *Mix & Go E. coli* from your favorite lab strains. **ZymoBroth™** growth medium is included.	T3001	Prepare up to 20 ml Competent Cells
***Mix & Go E. coli* Transformation Buffer Set** Includes all buffers for making up to 60 ml *Mix & Go E. coli* from your favorite lab strains. **ZymoBroth™** growth medium is **not** included.	T3002	Prepare up to 60 ml Competent Cells

Individual Kit Components	Catalog No.	Amount
ZymoBroth™	M3015-100 M3015-500	100 ml 500 ml
Wash Buffer (2X Stock)	T3001-2-10 T3001-2-30	10 ml 30 ml
Competent Buffer (2X Stock)	T3001-3-10 T3001-3-30	10 ml 30 ml
Dilution Buffer	T3001-4-20 T3001-4-60	20 ml 60 ml

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