ZYMO RESEARCH

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INSTRUCTION MANUAL

Mix & Go E. coli Transformation Kit & Buffer Set

Catalog Nos. **T3001** & **T3002**

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.

Contents

ZYMO RESEARCH CORP.

Toll Free: 1-888-882-9682 • Fax: 1-949-266-9452 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

ZymoBroth™ is included in the *Mix & Go E. coli* Transformation Kit (T3001).

ZymoBroth™ is not included in the *Mix & Go E. coli* Transformation Buffer Set (T3002).

Product Contents

	***Mix & Go E. coli* Transformation Kit** Includes all buffers for making up to 20 ml *Mix & Go E. coli*. **ZymoBroth™** growth medium is included. **T3001** (20 ml)	***Mix & Go E. coli* Transformation Buffer Set** Includes all buffers for making up to 60 ml *Mix & Go E. coli*. **ZymoBroth™** growth medium is not included. **T3002** (60 ml)	**Storage Temp.**
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
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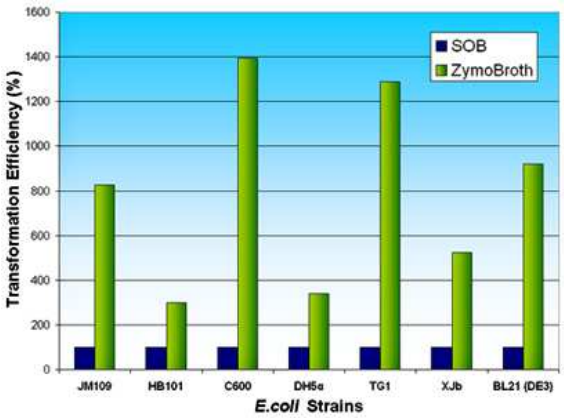
Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Product Description

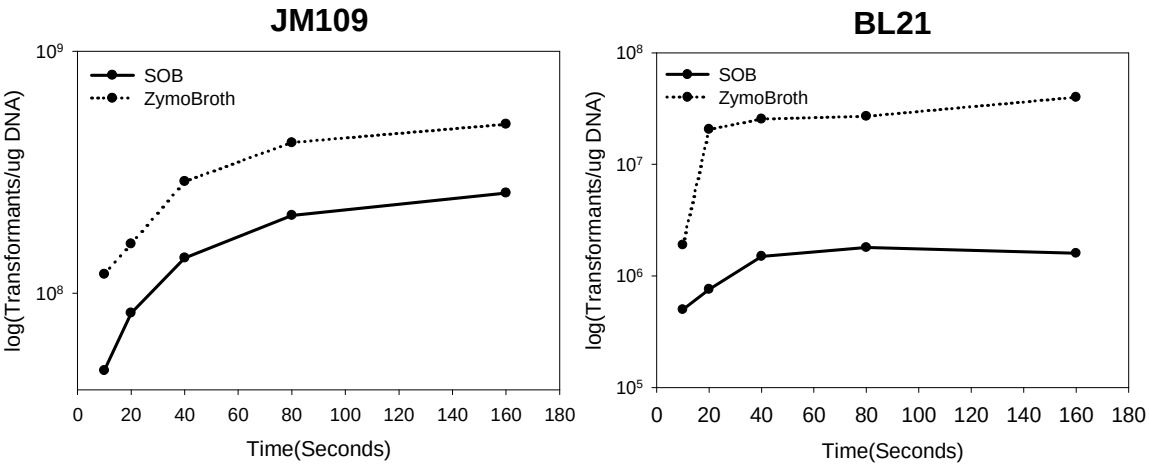
The *Mix & Go E. coli* Transformation Kit & Buffer Set are simple methods for generating *Mix & Go* chemically competent *E. coli* for rapid, reliable and highly efficient DNA transformation. The methods eliminate the requirement of heat-shocking and related procedures. Instead, transformation can be 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).



ZymoBroth™ medium dramatically increases the transformation efficiencies of a broad range of *E. coli* strains. The figure to the left shows the relative transformation efficiencies for different *E. coli* strains generated using SOB and ZymoBroth™. Generally, ZymoBroth™ medium enhances *E. coli* transformation efficiencies better for difficult to transform strains.

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

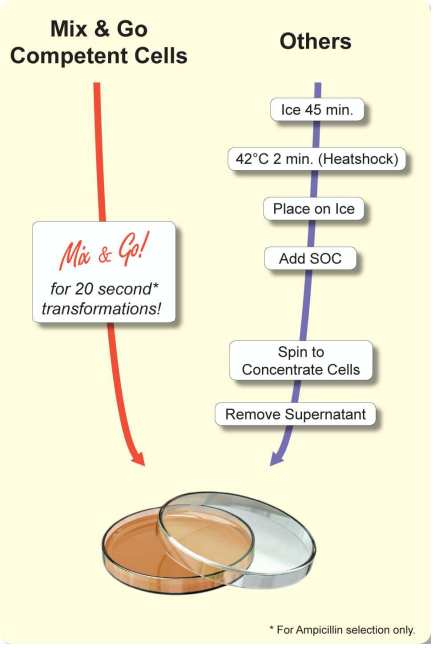
Note -™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Not intended for use in diagnostic procedures. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.



***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. 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

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

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℃. It is important that each ste p of the following procedure should be done on ice or at 0-4℃.

It is important that Steps 2-5 be performed 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.

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.

* 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.

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 below.

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 (above) 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).

*DH5α is a trademark of Life Technologies and XL10 Gold is a trademark of Stratagene.

Appendix

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 $MgCl_2$
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.

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.	Kit 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

For Individual Sale	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

Premade Mix & Go Competent Cells from Zymo Research

Product	Description	Kit Size	Cat No.
JM109 *Mix & Go* Competent Cells	General cloning, blue-white selection, plasmid isolation, healthy strain, transformation efficiency > 10^8. Genotype: 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	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips)	**T3003** **T3005**
Zymo 5α *Mix & Go* Competent Cells (Same as DH5α)	General cloning, blue-white selection, plasmid isolation (slow growth, certain plasmids not stable), transformation efficiency > 10^8. Genotype: F$^-$φ80dlacZΔM15 Δ(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(r_k^- m_k^+ phoA supE44 λ$^-$ thi-1 gyrA96 relA1	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips) 96 x 50 µl aliquots (PCR plates)	**T3007** **T3009** **T3010**
HB101 *Mix & Go* Competent Cells	General cloning, plasmid isolation, transformation efficiency > 10^8. Genotype: F-Δ(gpt-proA)62 leuB6 supE44 ara-14 galkK2 lacY1 Δ(mcrC-mrr) rpsL20 (Str^r) xyl-5 mtl-1 recA13	10 x 100 µl aliquots (10 tubes)	**T3011**
C600 *Mix & Go* Competent Cells	General cloning, plasmid isolation, transformation efficiency > 10^8. Genotype: F$^-$[e14-(McrA$^-$) or e14$^+$(mcrA$^+$] thr- 1leuB6 thi- 1 lacY1 supE44 rfbD1 fhuA21; the original C600 is EcoKr$^+$m$^+$ McrBC$^+$(2,3)	10 x 100 µl aliquots (10 tubes)	**T3015**
TG1 *Mix & Go* Competent Cells	General cloning, blue-white selection, plasmid isolation, transformation efficiency > 10^8. Genotype: F'traD36 lacI^{q}Δ(lacZ) M15 proA$^+$B$^+$ /supE Δ(hsdM-mcrB)5 (r_k - m_k - McrB$^-$) thi Δ(lac-proAB)	10 x 100 µl aliquots (10 tubes)	**T3017**
Zymo 10B *Mix & Go* Competent Cells (Same as DH10B)	For general cloning, blue-white selection, plasmid isolation. Transformation efficiency > 10^8. Ideal for cDNA generation and library construction. Genotype: F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG λ-	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips)	**T3019** **T3020**
XJa Autolysis™ *Mix & Go* Competent Cells	JM109 with chromosomally inserted λ lysozyme gene inducible by arabinose. Genotype: *E. coli* K recA1 supE44 endA1 hsdR17 (r_k^-,m_k^+) gyrA96 relA1 thi mcrAΔ(lac-proAB)Δ araB:: λR, cat F'[traD36 proAB+ lacI^q lacZ ΔM15]	10 x 100 µl aliquots (10 tubes)	**T3021**
XJb (DE3) Autolysis™ *Mix & Go* Competent Cells	BL21(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysogen encodes chromosomallyencoded 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. Genotype: E. coli B F- ompT hsdSB(rB- mB-) gal dcm+ araB::R,cat λDE3	10 x 100 µl aliquots (10 tubes)	**T3051**

Other E. coli Related Products from Zymo Research

Rattler™ Plating Beads	Sterile 5 mm glass plating beads are convenient and easy to use. No flaming required. Spread cells evenly over the entire surface of the culture plate.	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.*