INSTRUCTION MANUAL

Mix & Go 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*

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**Product Contents**

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<th>Format</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix &amp; Go Competent E. coli Cells</td>
<td>-</td>
</tr>
<tr>
<td>10 x 100 µl Single-Tube Aliquots</td>
<td>&lt; -70°C</td>
</tr>
<tr>
<td>96 x 50 µl Aliquots (12 x 8-Tube Strips)</td>
<td>-</td>
</tr>
<tr>
<td>96 x 50 µl Aliquots (96-well PCR plates)</td>
<td>-</td>
</tr>
<tr>
<td>Instruction Manual</td>
<td>1</td>
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<tr>
<td>Manual</td>
<td>1</td>
</tr>
<tr>
<td>Manual</td>
<td>1</td>
</tr>
</tbody>
</table>

Note – Chemically Mix & Go 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**

*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 (12x8- tube strips) of 50 µl/tube.

*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) was used for transformation and the data are the averages of three individual experiments.

*Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.*
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 3, Notes Section 2 and 4 regarding Incubation Time & Outgrowth).
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 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 *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.

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 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.

**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.
Genotypes

JM109
F' traD36 lacΔ(lacZ)M15 pro A+ B+ / e14- (McrA ) Δ(lac-proAB)thi gyrA96 (Nal') endA1 hsdR17(λr- m- )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., Agt11. Can be used for M13 cloning/sequencing and blue/white screening.

XJa
recA1 supE44 endA1 hsdR17 (λr- m- ) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB.: ΔR, cat F'[traD36 proAB+ lacIΔ(lacZ)M15]

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., Agt11. Can be used for M13 cloning/sequencing and blue/white screening.

XJa(DE3)
recA1 supE44 endA1 hsdR17 (λr- m- ) gyrA96 relA1 thi mcrA Δ(lac-proAB) ΔaraB.: ΔR, cat F'[traD36 proAB+ lacIΔ(lacZ)M15] DE3

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., Agt11. Can be used for M13 cloning/sequencing and blue/white screening.

DH5 Alpha
F-φ80lacZΔM15 Δ(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(λr- m- ) 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 galK2 lacY1 Δ(mcrC-mrr) rpsL20 (Str') xyl-5 mtl-1 recA13 (4)

TG1
F' traD36 lacIΔ(lacZ) M15 proA+B+ /supE Δ(hsdM-mcrB)5 (λr- m- McrB ) thi Δ(lac-proAB)

References:
**Ordering Information for Premade Mix & Go Competent Cells**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Cat. No.</th>
<th>Size*</th>
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</thead>
<tbody>
<tr>
<td>JM109</td>
<td>For general cloning, blue-white selection, plasmid isolation. Healthy strain w/ transformation efficiency &gt; 10⁸.</td>
<td>T3003</td>
<td>10 x 100 µl</td>
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<tr>
<td></td>
<td></td>
<td>T3005</td>
<td>96 x 50 µl (12 x 8-Tube Strips)</td>
</tr>
<tr>
<td>DH5 Alpha</td>
<td>For general cloning, blue-white selection, plasmid isolation. Slow growth w/ certain plasmids not stable. Transformation efficiency &gt; 10⁸.</td>
<td>T3007</td>
<td>10 x 100 µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3009</td>
<td>96 x 50 µl (12 x 8-Tube Strips)</td>
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<tr>
<td></td>
<td></td>
<td>T3010</td>
<td>96 x 50 µl (PCR Plates)</td>
</tr>
<tr>
<td>HB101</td>
<td>For general cloning, plasmid isolation. Transformation efficiency &gt; 10⁸.</td>
<td>T3011</td>
<td>10 x 100 µl</td>
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<tr>
<td></td>
<td></td>
<td>T3013</td>
<td>96 x 50 µl (12 x 8-Tube Strips)</td>
</tr>
<tr>
<td>TG1</td>
<td>For general cloning, blue-white selection, plasmid isolation. Transformation efficiency &gt; 10⁸.</td>
<td>T3017</td>
<td>10 x 100 µl</td>
</tr>
<tr>
<td>Zymo 10B (Same as DH10B)</td>
<td>For general cloning, blue-white selection, plasmid isolation. Transformation efficiency &gt; 10⁸. Ideal for cDNA generation and library construction.</td>
<td>T3019</td>
<td>10 x 100 µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3020</td>
<td>96 x 50 µl (12 x 8-Tube Strips)</td>
</tr>
<tr>
<td>XJa Autolysis™</td>
<td>JM109 w/ chromosomally inserted λ lysozyme gene that is inducible by arabinose.</td>
<td>T3021</td>
<td>10 x 100 µl</td>
</tr>
<tr>
<td>XJb (DE3) Autolysis™</td>
<td>BL21(DE3) with chromosomally inserted λ lysozyme gene inducible by arabinose. DE3 lysozen 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.</td>
<td>T3051</td>
<td>10 x 100 µl</td>
</tr>
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</table>

**Popular E. coli Related Products from Zymo Research**

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Kit Size</th>
<th>Cat No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mix &amp; Go E. coli Transformation Kit</td>
<td>Includes all buffers for making up to 20 ml Mix &amp; Go chemically competent E. coli. ZymoBroth™ medium is included.</td>
<td>20 ml</td>
<td>T3001</td>
</tr>
<tr>
<td>Mix &amp; Go E. coli Transformation Buffer Set</td>
<td>Includes all buffers for making up to 60 ml Mix &amp; Go chemically competent E. coli. ZymoBroth™ medium is not included.</td>
<td>60 ml</td>
<td>T3002</td>
</tr>
<tr>
<td>ZymoBroth™</td>
<td>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).</td>
<td>100 ml</td>
<td>M3015-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ml</td>
<td>M3015-500</td>
</tr>
<tr>
<td>Rattler™ Plating Beads</td>
<td>Sterile 5 mm plating beads are convenient and easy to use. No flaming required. Spread cells evenly over the entire culture plate surface.</td>
<td>1 Bottle</td>
<td>S1001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Bottles</td>
<td>S1001-5</td>
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*Bulk quantities are available upon request. Please contact: busdev@zymoresearch.com or call 1-888-882-9682 for assistance.