invitrogen™

Competent Cell Selection Kit

Cat. No. A10469

Size: 2 vials per cell type (2 × 50 µl)
Store at –80°C.

Kit Components

Sufficient reagents are provided for 2 transformations per cell type (50 µl/reaction).

Cells	Vial Cap Color	Amount	Storage
OmniMAX™ 2-T1^R Cells	Pink	2 × 50 µl	–80°C
Mach1™-T1^R Cells	Blue	2 × 50 µl	–80°C
Stbl3™ Cells	Clear	2 × 50 µl	–80°C
TOP10 Chemically Competent *E. coli* Cells	Purple	2 × 50 µl	–80°C

Additional Components

S.O.C. Medium	7 ml	Room temperature or +4°C
pUC19 Control DNA (10 pg/µl)	50 µl	–80°C

Description

The Competent Cell Selection Kit contains a set of four different strains of chemically competent *E. coli* cells in convenient One Shot® formats. Each single-use vial of cells keeps the cells stable and ready to use, with no extra pipetting steps or freeze-thaw cycles. This selection kit includes S.O.C. media and pUC 19 control DNA, and is designed to allow you to set up side-by-side reactions for comparing cell performance.

One Shot® OmniMAX™ 2-T1^R Chemically Competent *E. coli* provides high transformation efficiency (>5 x 10^9 cfu/µg DNA) and offers several additional features that make it an ideal strain for use in most cloning applications:

- Δ(*ccd*AB) for sensitivity to the toxic effects of the *ccd*B gene product, allowing negative selection of vectors containing the *ccd*B gene
- Elimination of *mcr*A, *mrr*, *mcr*BC, and *hsd*RMS restriction systems to allow construction of more representative genomic libraries (Blumenthal, 1989; Grant *et al.*, 1990)
- *ton*A genotype to confer resistance to T1 and T5 phage

One Shot® Mach1™-T1^R Chemically Competent *E. coli* is modified from the wild-type W strain (ATCC #9637, S. A. Waksman) and has a faster doubling time compared to other standard cloning strains. With Mach1™-T1^R cells, you can visualize colonies 8 hours after plating on ampicillin-selective plates. You can also prepare plasmid DNA 4 hours after inoculating a single, overnight-grown colony in the selective media of choice. Note that this feature is not limited to ampicillin selection. Additional key features of the Mach1™-T1^R *E. coli* strain include:

- *lac*ZΔM15 for blue/white color screening of recombinants
- *hsd*R mutation for efficient transformation of unmethylated DNA from PCR applications
- Δ*rec*A1398 mutation for reduced occurrence of homologous recombination in cloned DNA
- *end*A1 mutation for increased plasmid yield and quality
- *ton*A mutation to confer resistance to T1 and T5 phage

One Shot® Stbl3™ Chemically Competent *E. coli* is derived from the HB101 *E. coli* strain and is recommended for use when cloning unstable inserts such as lentiviral DNA containing direct repeats (*e.g.*, Invitrogen's ViraPower™ Lentiviral Expression Kits). The transformation efficiency of One Shot® Stbl3™ chemically competent cells is greater than 1 × 10^8 cfu/µg DNA. **Note:** This strain is *end*A1+.

One Shot® TOP10 Chemically Competent *E. coli* is provided at a transformation efficiency of 1 × 10^9 cfu/µg supercoiled DNA and are ideal for high-efficiency cloning and plasmid propagation. They allow stable replication of high-copy number plasmids. The genotype of TOP10 Cells is similar to the DH10B™ strain.

General Guidelines for Handling Competent Cells

Handle competent cells gently, as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently. **Do not mix cells by pipetting**.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per µg of plasmid DNA:

$$\frac{\text{\# of colonies}}{\text{10 pg transformed DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{\text{300 µl total transformation volume}}{\text{X µl plated}} \times \text{Dilution factor} = \frac{\text{\# transformants}}{\mu\text{g plasmid DNA}}$$

For example, if transformation of 10 pg of pUC19 DNA yields 40 colonies when 25 µl of a 1:10 dilution is plated, then the transformation efficiency is:

$$\frac{\text{40 colonies}}{\text{10 pg DNA}} \times \frac{10^6\ \text{pg}}{\mu\text{g}} \times \frac{\text{300 µl total volume}}{\text{25 µl plated}} \times 10 = 4.8 \times 10^8$$

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One Shot® OmniMAX™ 2-T1R Chemically Competent *E. coli*

Vial Cap Color: Pink

Genotype

F′ {*pro*AB+ *lac*Iq *lac*ZΔM15 *Tn*10(TetR) Δ(*ccd*AB)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80(*lac*Z)ΔM15 Δ(*lac*ZYA-*arg*F) U169 *end*A1 *rec*A1 *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A *pan*D

Information for European Customers

The OmniMAX™ 2-T1R strain is genetically modified and carries the F′ episome containing *pro*AB+ *lac*Iq *lac*ZΔM15 *Tn*10 Δ(*ccd*AB). As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Caution

This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Guidelines for Cell Use

One Shot® OmniMAX™ 2-T1R cells are *lac*Iq and require IPTG to induce expression from the *lac* promoter. Spread 40 µl of 100 mM IPTG on top of the agar. Let the IPTG diffuse into the agar for approximately 1 hour. If blue/white screening is required to select for transformants, spread 40 µl of 40 mg/ml X-Gal in dimethylformamide in addition to IPTG on top of the agar. Let the X-Gal and IPTG diffuse into the agar for approximately 1 hour.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium to room temperature.
- Spread IPTG or IPTG and X-Gal onto LB agar plates containing antibiotic, if desired.
- Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

Transformation Procedure

We recommend including the pUC19 control plasmid DNA supplied with the kit in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot® OmniMAX™-T1R chemically competent cells for each transformation.
2. Add 1 to 5 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.** If you are transforming the pUC19 control, add 1 µl (10 pg) into a separate vial of One Shot® cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place on ice for 2 minutes.
6. Add 250 µl of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Before plating, dilute the transformation mix 1:50 into LB Medium (*e.g.* remove 10 µl of the transformation mix and add to 490 µl of LB Medium).
9. Spread 25-100 µl of the diluted transformation mix on a pre-warmed selective plate. Store the remaining undiluted and diluted transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

One Shot® Mach1™-T1R Chemically Competent *E. coli*

Vial Cap Color: Blue

Genotype

F- ϕ80(*lac*Z)ΔM15 Δ*lac*X74 *hsd*R($r_K^- m_K^+$) Δ*rec*A1398 *end*A1 *ton*A

Information for Non-U.S. Customers

For European Customers

The Mach1™-T1R *E. coli* strain is genetically modified to carry the *lac*ZΔM15 *hsd*R *lac*X74 *rec*A *end*A *ton*A genotype. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

For All Non-U.S. Customers

The parental strain of Mach1™-T1R *E. coli* is the non-K-12, wild-type W strain (ATCC #9637, S. A. Waksman). Although the parental strain is generally classified as Biosafety Level 1 (BL-1), we recommend that you consult the safety department of your institution to verify the Biosafety Level.

Guidelines for Cell Use

One Shot® Mach1™-T1R cells do not require IPTG to induce expression from the *lac* promoter. If blue/white screening is required to select for transformants, spread 40 µl of 40 mg/ml X-Gal in dimethylformamide on top of the agar. Let the X-Gal diffuse into the agar for approximately 1 hour.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium to room temperature.
- Spread X-Gal onto LB agar plates containing antibiotic, if desired.
- **Important for Mach1™-T1R *E. coli* cells :** Warm the selective plates in a 37°C incubator for 30 minutes (use one plate per transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

Transformation Procedure

We recommend including the pUC19 control plasmid DNA supplied with the kit in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot® Mach1™-T1R Chemically Competent *E. coli* for each transformation.
2. Add 1 to 5 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.** If you are transforming the pUC19 control, add 1 µl (10 pg) into a separate vial of One Shot® cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove vial(s) from the 42°C bath and place on ice for 2 minutes.
6. Add 250 µl of room temperature S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 25-100 µl of the transformation mix on a **prewarmed** selective plate. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium (*e.g.* remove 100 µl of the transformation mix and add to 900 µl of LB Medium) and plate 25-100 µl.
9. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the plate(s) and incubate at 37°C. If you are using ampicillin selection, visible colonies should appear within 8 hours, and blue/white screening can be performed after 12 hours. If you are selecting transformants with an antibiotic other than ampicillin, incubate plates overnight.
11. Select overnight-grown colonies and analyze by plasmid isolation, PCR, or sequencing. For plasmid isolation, inoculate a single, overnight-grown colony in 2 ml of **prewarmed** selective media (*e.g.* LB + ampicillin, LB + kanamycin, LB + Zeocin™, *etc.*). For optimal results, we recommend inoculating as much of the single colony as possible. Shake at 37°C for 4 hours before isolating the plasmid.

One Shot® Stbl3™ Chemically Competent *E. coli*

Vial Cap Color: Clear

Genotype

F⁻ *mcr*B *mrr hsd*S20(r_B^-, m_B^-) *rec*A13 *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Str^R) *xyl*-5 λ⁻ *leu mtl*-1

Guidelines for Cell Use

Cells cannot be used for blue/white screening of plasmid inserts.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

Transformation Procedure

We recommend including the pUC19 control plasmid DNA supplied with the kit (10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot® Stbl3™ chemically competent cells for each transformation.
2. Add 1 to 5 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.** For the pUC19 control, add 10 pg (1 µl) of DNA into a separate vial of One Shot® cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 45 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
6. Add 250 µl of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 25-100 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
9. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the selective plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

One Shot® TOP10 Chemically Competent *E. coli*

Vial Cap Color: Purple

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G λ-

Guidelines for Cell Use

One Shot® TOP10 cells do not require IPTG to induce expression from the *lac* promoter. If blue/white screening is required to select for transformants, make sure that selective plates contain 50 µg/ml X-gal.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Equilibrate a water bath to 42°C.
- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Spread X-gal onto LB agar plates containing antibiotic, if desired.
- Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

Transformation Procedure

We recommend including the pUC19 control plasmid DNA supplied with the kit (10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot® TOP10 chemically competent cells for each transformation.
2. Add 1 to 5 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.** For the pUC19 control, add 10 pg (1 µl) of DNA into a separate vial of One Shot® cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 30 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
6. Aseptically add 250 µl of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 20-200 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium (*e.g.* remove 100 µl of the transformation mix and add to 900 µl of LB Medium) and plate 25-100 µl.
9. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the selective plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Rapid Transformation Procedure

This procedure is only recommended for transformations utilizing ampicillin selection. It is essential to pre-warm selective plates prior to spreading the transformed cells.

1. Centrifuge the ligation reactions briefly and place on ice.
2. Thaw, on ice, one 50 µl vial of One Shot® cells per transformation.
3. Pipet 1-5 µl of each ligation reaction into the vial of competent cells and mix by tapping gently. **Do not mix by pipetting up and down.** Store remaining ligation reactions at -20°C.
4. Incubate the cells on ice for 5 minutes.
5. Heat-shock the cells for 30 seconds at 42°C without shaking.
6. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
7. Immediately spread 50 µl of transformed cells on a pre-warmed selective plate containing 100 µg/ml ampicillin.
8. Incubate at 37°C overnight.
9. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Additional Products

The following products are available from Invitrogen. Visit our web site at www.invitrogen.com to order.

Product	Amount	Catalog no.
One Shot® OmniMAX™ 2 T1 Phage-Resistant Cells	20 reactions	C8540-03
One Shot® Mach1™-T1^R Chemically Competent *E. coli*	20 reactions	C8620-03
One Shot® Stbl3™ Chemically Competent *E. coli*	20 reactions	C7373-03
One Shot® TOP10 Chemically Competent *E. coli*	20 reactions	C4040-03
	10 reactions	C4040-10
	40 reactions	C4040-60

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

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