MultiShot™ FlexPlate Competent Cell Kits

Catalog Numbers C86812-01, C85812-01, C73812-01, C64812-01, C44812-01,and C40812-01

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ MultiShot™ FlexPlate Competent Cell Kits contain chemically competent *E. coli* cells pre-filled in a 96-well PCR plate to improve productivity when performing bacterial transformation. The FlexPlate format allows the plate to be separated into 8-well segments to provide flexibility in transformation throughput.

Contents and storage

Kit type	Cat. No.
MultiShot™ FlexPlate DH5α™ T1^R Competent Cells	C44812-01
MultiShot™ FlexPlate DH10B™ T1^R Competent Cells	C64812-01
MultiShot™ FlexPlate Mach1™ T1^R Competent Cells	C86812-01
MultiShot™ FlexPlate OmniMAX™ 2 T1^R Competent Cells	C85812-01
MultiShot™ FlexPlate Stbl3™ Competent Cells	C73812-01
MultiShot™ FlexPlate TOP10 Competent Cells	C40812-01

Contents	Amount	Storage
Chemically competent cells (20 µL well)	FlexPlate 96-well PCR plate	–80°C to –70°C
pUC19 vector, supercoiled (10 pg/ µL) [1]	50 µL	
S.O.C. medium	2 × 10 mL	Room temperature or 4°C
Adhesive foil seal	2	Room temperature

[1] In 5 mM Tris-HCl, 0.5 mM EDTA, pH 8

Genotype

Strain	Genotype
DH5α™ T1^R	F$^-$ Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17 (r_K^- m_K^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A
DH10B™ T1^R	F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 *lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(araleu)7697 *gal*U *gal*K *rps*L *nup*G λ$^-$ *ton*A
Mach1™ T1^R	F$^-$ Φ80*lac*ZΔM15 Δ*lac*X74 *hsd*(r_K^- m_K^-) Δ*rec*A1398 *end*A1 *ton*A
OmniMAX™ 2 T1^R	F$^-$ {*pro*AB *lac*I^q *lac*ZΔM15 *Tn*10(Tet^R) Δ(*ccd*AB)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *ton*A *pan*D
Stbl3™	F$^-$ *mrrhsd*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
TOP10	F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(araleu)7697 *gal*U *gal*K *rps*L20(Str^R) *end*A1 *nup*G

Required materials not provided

- 42°C water bath or heat block (High Efficiency Transformation Protocol only)
- 37°C incubator
- LB agar plates with appropriate antibiotic (e.g., ampicillin for transformation controls)
- Ice bucket with ice

Procedural guidelines

- **IMPORTANT!** Do not transform chemically competent cells by electroporation. The salt content of the buffer will cause arcing and kill the cells.
- Be extremely gentle when working with competent cells. Competent cells are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Start transformation immediately after thawing the cells on ice. Mix cells by gentle stirring with a pipette tip; do not mix by pipetting up and down.
- Avoid freeze-thaw cycles for pUC19 control DNA. Prepare aliquots of pUC19 control DNA and store at –80°C.
- Practice sterile technique when handling S.O.C. medium to avoid contamination.
- If using only a portion of the plate, separate the required number of segments for your experiment by scoring the plate with a sharp edged implement (e.g., razor blade, scissor tip) within the –80°C freezer, then carefully bend and separate the required segments. Store the unused portion of the FlexPlate at –80°C.

  IMPORTANT! Use extreme caution when working with sharp implements.
- Perform a transformation control to test the transformation efficiency of the competent cells in the kit. See "Calculate transformation efficiency" on page 5 for details.

Transformation procedures

Two protocols are provided to transform MultiShot™ FlexPlate Competent Cells. Consider the following factors when choosing which protocol to use.

If you wish to...	Then use the...
maximize the number of transformants obtained	"High Efficiency Transformation Protocol"
use an antibiotic other than ampicillin to select for your DNA	
obtain transformants as quickly as possible	"Rapid Transformation Protocol" on page 4

High Efficiency Transformation Protocol

The High Efficiency Transformation Protocol is used to achieve the highest efficiency when transforming MultiShot™ competent cells with your DNA of interest.

Before you begin

- Warm S.O.C. medium to room temperature if necessary.
- Pre-warm LB selection plates in a 37°C incubator **for at least 30 minutes**.
- Pre-heat water bath or heat block to 42°C.

Prepare cells

1. Thaw the FlexPlate or FlexPlate segments on ice for 2–5 minutes.
2. Carefully remove the seal from the FlexPlate or FlexPlate segments.

Transform cells

1. Add ≤2 µL of DNA directly into each well.

   Note: For optimal results, do not add more than ≤2 µL (10% of the cell volume) of DNA.

2. Mix the cells by gently stirring the mixture with a pipette tip three times. **Do not** mix by pipetting up and down.
3. Seal the FlexPlate with the supplied adhesive seal and incubate the cells on ice for 30 minutes.

   Note: If using FlexPlate segments, cut a section from the adhesive seal sufficient to cover the wells. Each segment requires a section of 1 cm to seal the wells.

4. Incubate the FlexPlate wells at 42°C in a water bath or on a heat block. Do not mix or shake.

Strain	Incubation time
DH5α™ T1^R, DH10B™ T1^R, Mach1™ T1^R, OmniMAX™ 2 T1^R, and TOP10	30 seconds
Stbl3™	45 seconds

5. Incubate the FlexPlate wells on ice for 2 minutes.

6. Place the FlexPlate wells on the benchtop and remove the adhesive seal.

7. Add 140 µL of S.O.C. medium to each FlexPlate well.

   Note: S.O.C. is a rich medium; good sterile technique must be practiced to avoid contamination.

8. Seal the FlexPlate wells with the adhesive seal.

9. Incubate the FlexPlate wells at 37°C for 1 hour. **No shaking**.

10. Dilute transformation reaction with S.O.C. medium as needed.

Strain	Dilution factor
DH5α™ T1^R, DH10B™ T1^R, Mach1™ T1^R, OmniMAX™ 2 T1^R, and TOP10	1:10
Stbl3™	No dilution

Note: Ensure the transformation reaction is thoroughly mixed before dilution.

Plate transformed cells

1. Plate 50–100 µL of cells from each transformation reaction on pre-warmed LB selection plates, then invert the plates and incubate overnight at 37°C.

   Note: Ensure the transformation reaction is thoroughly mixed before plating.

2. Select colonies for further analysis by plasmid isolation, PCR, or sequencing.

Rapid Transformation Protocol

The Rapid Transformation Protocol is used to obtain transformants as quickly as possible, and is only compatible with vectors with ampicillin resistance. Using this protocol to transform MultiShot™ competent cells can result in a 1-log reduction in transformation efficiency.

Before you begin

Pre-warm LB selection plates in a 37°C incubator long enough to ensure the plates reach 37°C (**a minimum of 1 hour**). Prepare one plate for each transformation.

Transform competent cells

1. Thaw the FlexPlate or FlexPlate segments on ice for 2–5 minutes.
2. Add ≤2 µL of DNA directly into each well.
3. Mix the cells by gently stirring the mixture with a pipette tip three times. **Do not** mix by pipetting up and down.
4. Seal the wells with the adhesive seal and incubate the cells on ice for 5 minutes.
5. Immediately plate 5–20 µL of cells from each transformation reaction on pre-warmed LB selection plates, then invert the plates and incubate overnight at 37°C.

Perform transformation control

1. Prepare competent cells as described in "Prepare cells".
2. Add 1 µL of the pUC19 control DNA to thawed cells, then follow the instructions in "Transform cells" starting from step 2.
3. Plate 30–50 µL of cells on pre-warmed LB agar plates containing 100 µL/mL ampicillin (see "Plate transformed cells" for detailed instructions).

Calculate transformation efficiency

Use the following formula to calculate transformation efficiency in terms of colony forming units per µg of DNA (CFU/µg).

$$\text{Transformation efficiency (CFU/µg)} = \frac{\text{\# of colonies (CFU)}}{\text{µg transformed DNA}} \times \frac{\text{Transformation volume (µL)}}{\text{Plating volume (µL)}} \times \text{Dilution factor of transformed cells}$$

Use the following formula to determine the amount of DNA used for transformation for ligation reactions.

$$\text{µg transformed DNA} = \frac{\text{DNA in ligation reaction (µg)}}{\text{Ligation volume (µL)}} \times \text{Dilution factor of ligation product} \times \text{Volume of ligation product used for transformation}$$

Example of transformation efficiency calculation

50 ng of DNA is ligated in a 20 µL reaction. After ligation, the reaction is diluted 2-fold and 5 µL of the diluted ligation mixture is added to 100 µL of competent cells for transformation.

$$\text{µg transformed DNA} = \frac{0.5\ \text{µg}}{20\ \text{µL}} \times 2 \times 5\ \text{µL} = 0.025\ \text{µg}$$

After transformation, the cell suspension is diluted 5-fold and 200 µL of the diluted cells are plated. 300 colonies are formed after overnight incubation.

$$\text{Transformation efficiency (CFU/µg)} = \frac{300\ \text{CFU}}{0.025\ \text{µg}} \times \frac{100\ \text{µL}}{200\ \text{µL}} \times 5 = 3 \times 10^4\ \text{CFU/µg}$$

Troubleshooting

For troubleshooting help on competent cells, go to **thermofisher.com/compcellsupport**

Observation	Possible cause	Recommended action
Few or no colonies	Problem with competent cells.	Carry out the pUC19 control DNA transformation to obtain information about the performance of the cells.
	Problem with the antibiotic in the plate.	Confirm that the correct selection antibiotic and concentration was used in the LB agar plate.
	Using a vector with a selection marker that is not ampicillin when performing the Rapid Transformation Protocol.	If the antibiotic selection marker for the vector being transformed is not ampicillin, use the High Efficiency Transformation Protocol.
	LB plate used for growing colonies insufficiently warmed.	Ensure that the LB agar plates are pre-warmed to 37°C prior to plating. Incubate LB agar plates for at least 30 minutes if using the High Efficiency Transformation Protocol, and for at least 1 hour if using the Rapid Transformation Protocol.
Transformants contain incorrect or truncated DNA inserts	Instability in DNA being cloned.	Use Stbl3™ competent cells for transformation to prevent plasmid recombination.
	DNA mutation.	Mutations may have occurred during plasmid propagation in transformed cells. Pick a sufficient number of colonies for representative screening by sequencing. If all colonies carry the same mutation, it may have originated from the original template.

Accessory products

Product	Cat. No.
LB Broth, 500 mL	10855021
LB Broth Base, powder 500 g	12780052
LB Agar, powder 500 g	22700025
X-gal, 100 mg	15520034
S.O.C. Medium, 10 × 10 mL	15544034
Adhesive PCR Plate Foil	AB0626

For European customers

The Mach1™ T1 Phage-Resistant Chemically Competent *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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008

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Revision	Date	Description
A	11 April 2018	New document

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