

Competent Cells

Product Use Limitation & Warranty

This product is intended to be used for life science research $\frac{9}{4}$ of all products in the manner described in our protocol. The particular use. Should any product fail to perform satisfactorily



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1 Minute E. coli Transformation **SOC Eliminated** Revolution

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General Information

ECOS™ Competent Cells are chemically competent cells, which were prepared using our proprietary process to make the cells highly efficient for immediate DNA uptake. The transformation process only takes 1 minute to finish and the steps are simplified. There are no other competent cells that offer faster and easier transformation than ECOS™.

Kit Contents

- (1) ECOS[™] Competent Cells.
- (2) pUC19 Control Plasmid (5 μl, 10⁻⁴ μg/μl).
- (3) ECOS[™] One-minute / Six-minute Patented Protocol

Shipping Conditions

The shipping temperature is maintained within at -65±5 °C.

Storage and Expiration

ECOS™ Competent Cells must be sotred at -70 °C to -80 °C. Subsequent freeze-thaw cycles will reduce transformation efficiency. If high efficiency is essential for the experiment, DO NOT use aliquots that have gone through serveral freeze-thaw cycles. The efficiency of ECOS™ Competent Cells is good for at least 1 year with proper storage.

Cat. No.	Product Name	Compatible to (strain)	Efficiency (cfu/µg)	Quantity
FYE610-10VL	ECCOTM V	E. coli	- 5 - 409	100 μ l × 10 via l s
FYE610-80VL	ECOS™ X	DH5α	>5x10 ⁹	100 μ l × 80 vials
FYE707-10VL				100 μ l × 10 via l s
FYE707-80VL	Economic ECOS™ 9-5		>5x10 ⁷	100 μ l × 80 vials
FYE708-10VL		E. coli		100 μ l × 10 vials
FYE708-80VL	ECOS™ 9 - 5	JM109	>1x10 ⁸	100 μ l × 80 vials
FYE709-10VL	0 = F000TM 0. F		- F: 408	100 μ l × 10 vials
FYE709-80VL	Super ECOS™ 9-5		>5x10 ⁸	100 μ l × 80 vials
FYE507-10VL				100 μ l × 10 via l s
FYE507-80VL	Economic ECOS™ 10B	E. coli	>5x10 ⁷	100 μ l × 80 vials
FYE508-10VL	ECOS™ 10B	DH10B	>1x10 ⁸	100 μ l × 10 vials
FYE508-80VL	ECO3 10B		~1X10°	100 μl × 80 vials
FYE807-10VL	F	E. coli	- 4 - 4 0 7	100 μ l × 10 vials
FYE807-80VL	Economic ECOS™ 2163	GM2163	>1x10 ⁷	100 μ l × 80 vials

Note: pUC19 is used for the evaluation of transformation efficiency.

OEM Service

In order to meet customer needs, we provide OEM of E. coli competent cells using our ECOS™ technology. Any E. coli strain can be prepared to become ECOS™ competent cells which can be transformed using our patented protocols. Customers will only need to send the E. coli strain to us along with information of its genotype and note for basic culture conditions. Typically, the total procedure can be finished in 30-60 days. However, a minimum order is required for this service.

Genotypes and Applications

Product	Genotype	Application
ECOS™ Blue	recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F'proAB lacl ^α ZΔM15 Tn10 (Tet')].	Suitable for blue-white color screening and routine cloning application.
ECOS™21	$F^{\text{-}} \textit{ompT} \textit{hsdS}_{\beta}(r_{\beta} 'm_{\beta} ') \textit{dcm} \textit{gal} \lambda(DE3)$	Appropriate host for recombinant protein expression using T7-based expression vectors.
ECOS™ 101	endA1 recA1 relA1 gyrA96 hsdR17($r_{\rm k}$, $m_{\rm k}$) phoA supE44 thi-1 Δ (lacZYA-argF)U169 Φ 80 Δ (lacZ)M15 F	Suitable for cloning with large plasmids and cDNA library construction, and also allow blue-white colony selection.
ECOS™ 9-5	e14"($McrA^-$) recA1 endA1 gyrA96 thi-1 hsdR17($r_{\rm K}^ m_{\rm K}^+$) supE44 relA1 Δ (lac-proAB) [F' traD36 proAB lacl $^{\rm C}$ 2 Δ M15]	Appropriate for blue-white color and robotic screening. It is a fast growing strain, forming visible colonies within 8 ~ 10 hours.

Product	Genotype	Application
ECOS™ 10B	F ⁻ endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80/acZΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS- mcrBC) λ ⁻	Suitable for the propagation of large DNA library clones, maintenance of large plasmids, cloning of methylated cytosine or adenine containing DNA, for blue/white selection.
ECOS™2163	F ⁻ ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 rpsL136 dam13::Tn9 xylA5 mtl-1 thi-1 mcrB1 hsdR2	Suitable for the propagation of plasmid or cloned DNA to be cut with Dam or Dcm-sensitive restriction enzymes, this strain is both Dam- and Dcm

Related Products

Cat. No.	Product Name	Description	Quantity
FYO001-100G	Plating Beads (4 mm)	Spread competent cells for <i>E. coli</i> and yeast transformation	100 g

Transformation Protocol

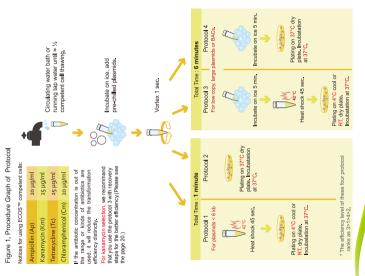
Heat shock / cold plating (protocol 1 & 3, please refer to Fig.1 on page 10)

- 1. Prepare the "dried" selective LB agar plates at 4 °C or room temperature for the plating step.
- 2. Thaw competent cells (typically, 100 µl) at room temperature in a water bath with circulating water or holding the tube under the running tap water for ~20 seconds until 1/3-1/2 volume is thawed. (If many transformations are carried out at once, keeping the competent cells on ice will generate better efficiency.)
- Add DNA (pre-chilled on ice, volume should be ≤ 5% of competent cells) immediately vortex for 1 second.
- 4. -For protocol 3> Keep the tubes on ice for 5 mins will slightly increase the efficiency. If many transformations are carried out at a time, this step will facilitate the process and make the overall efficiency more uniform.
- Heat shock the cells in a water bath at 42 °C for 15~45 seconds.
 (For different heat shock duration, please refer to Q8 on page 13.)
- Plate the cells using Plating Beads (Cat. No.: FYO001-100G) onto a pre-chilled (4 °C) and dried selective LB agar plate (LB+antibiotics).
- 7. Incubate the plates at 37 °C (8~16 hours for ECOS™ 9-5, 12~16 hours for others).

Non-heat shock / warm plating (protocol 2 & 4, please refer to Fig.1 on page 10)

- 1. Prewarm the "dried" selective LB agar plates at 37 °C incubator.
- 2. Thaw competent cells (typically, 100 μl) at room temperature in a water bath with circulating water or holding the tube under the running tap water for ~20 seconds until 1/3~1/2 volume is thawed. (If many transformations are carried out at once, keeping the competent cells on ice will generate better efficiency.)
- Add DNA (pre-chilled on ice, volume should be ≤ 5% of competent cells) immediately vortex for 1 second.

- 4. <For protocol 4> Keep the tubes on ice for 5 mins will slightly increase the efficiency. If many transformations are carried out at a time, this step will facilitate the process and make the overall efficiency more uniform.
- 5. Plate the cells using Plating Beads (Cat. No.: FYO001-100G) onto the pre-warmed and dried selective LB agar plates (LB+antibiotics).
- 6. Incubate the plates at 37 °C (8~16 hours for ECOS™ 9-5, 12~16 hours for others).

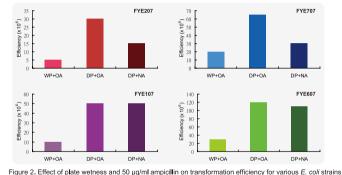


- 1. Efficiency test: Each batch of ECOS™ is checked rigorously to meet the product specifications as described in pages 3-4 (Items and Ordering Information) at the time of production.
- Calculation of transformation efficiency is as follows:
- a. Equation for transformation efficiency = transformed colonies (transformants)/µg of plasmid. b. Example:
 - 100 μI of competent cells have been transformed with 10⁻⁶ μg of pUC19 plasmid. If 550 colonies are abserved on the selective plate. The transformation efficiency is: 550/10⁻⁶= 5.5x10⁸ transformants / µg of pUC19 plasmid.
- 2. Contamination test: Transformation is performed without plasmid. There should be no colony on the LB agar plate with 20~50 µg/ml of ampicillin.
- α-complementation test for ECOS™ Blue, ECOS™101, ECOS™ X, ECOS™ 9-5, ECOS™ 10B, ECOS™ 2163: To pass the test, the ratio of white colonies/ white & blue colonies of the tested plate should be less than 3%.

Q & A

- Q1: How much will the efficiency be reduced if ECOS[™] competent cells are thawed, dispensed and refrozen repeatedly?
- A1: If ECOS™ competent cells are thawed, dispensed in aliquots and refrozen within 3 min, the transformation efficiency will be 20~50% less than first time use.
- Q2: What's the advantage of thawing the cells with circulating water instead of still water?
- A2: To thaw the cells with circulating water can increase the transformation efficiency by 1.5~3 times.

Q3: Do temperature, wetness of plating beads and plates affect transformation efficiency? A3: The transformation efficiency is increased significantly when dried plating beads and plates are used.



(FYE107, FYE207, FYE607, FYE707) using 1 min protocol. The results showed that using dried plate with old batch of ampicillin have a higher transformation efficiency.

- WP+OA; Wet plate with old batch (over 3 months from the date of opening) of ampicillin.
- Dried plate with old batch of ampicillin.
- Dried plate with fresh batch (within 3 months from the date of opening) of ampicillin.

- Q4: Do the storage temperature and thawing method affect the transformation efficiency?
- A4: Slow thawing caused by power shortage or unstable freezer will make the efficiency decreased. Therefore it is very important that the competent cells are stored at -70 °C at all time. Thawing the competent cells in water at room temperature yields better efficiency than thawing the cells on ice.
- Q5: Is there a difference in transformation efficiency between using plating beads and streaking loop?
- A5: Using plating beads gives higher transformation efficiency than using streaking loop.
- Q6: How to prepare dried selection plates?
- A6: During the process of making the agar plates, leave the freshly made plates in a laminar flow for 30~60 min to allow the moisture to be fully evaporated. Then store the plates at 4 °C (chilling & dry) or 37 °C (warm & dry) for more than 1 hour.
- Q7: Is one second vortex before 42 °C heat shock necessary for transformation?
- A7: One second vortex leads to more reliable transformation efficiency (1.2 times higher than mixed by finger tapping).
- Q8: Does the duration of heat shock affect transformation efficiency?
- A8: There is little difference in transformation efficiency within 15~45 seconds of heat shock. For most ECOS™ strains, 15~35 seconds of heat shock is optimal for transformation of plasmid < 6 Kb, 45 seconds heat shock is not optimal for plasmid < 6 Kb (may cause 1.2~2.5 times decrease, strain dependent) but is optimal for plasmid > 6 Kb.
- Q9: Is it necessary to change the transformation procedure for transforming *E. coli* with a large plasmid?
- A9: For large plasmid (> 6 Kb), 6 mins protocol should be used to significantly improves the efficiency (Fig. 1, Table 2).
- Q10: How to reduce the interference of the satellite colonies?
- A10: Using dried plating beads and plates with proper antibiotics at suitable concentrations. Fresh antibiotics are recommended.

Q11: Does the concentration of antibiotic in LB medium affect transformation efficiency?
A11: (1) For ampicillin

ECOS™ Blue, ECOS™ 101:

LB + 20 μg/ml fresh Amp. or 50 μg/ml old Amp. that has been stored for more than 3 months. If higher concentration is used, e.g. 50~100 μg/ml fresh Amp., the efficiency will be 3~10 times lower. Transformed colonies can be observed after 11~16 hours of incubation and the satellite colonies will start to appear after 18 hours.

ECOS™ 9-5 LB + 20 μg/ml fresh Amp. or 50 μg/ml old Amp. that has been stored for more than 3 months. If

higher concentration is used, e.g. 50~100 μg/ml fresh Amp., the efficiency will be 3~50 times lower. Transformed colonies can be observed after 8~10 hours of incubation and the satellite colonies will start to appear after 24 hours.

(2) For other antibiotics

LB + 20 μg/ml Kanamycin, 7.5 μg/ml Tetracycline, 20 μg/ml Chloramphenicol.
For plasmid size < 6 Kb, the efficiency of kanamycin selection is usually 3~10 times less than that of ampicillin selection. For plasmid size > 6 Kb, the efficiency of kanamycin selections is much lower than that of ampicillin. We recommend using the traditional protocol (with the recovery step) to enhance the efficiency. For more information about the relationship of antibiotic concentrations, protocol used, and efficiency levels, check the experimental data in Table 1 and 2.

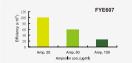


Figure 3. Effect of concentrations of ampicillin (fresh batch) on transformation efficiency using 1 min protocol with dried plate.

Amp. 20: 20 μg/ml ampicillin. Amp. 50: 50 μg/ml ampicillin.

Amp. 100: 100 μg/ml ampicillin.

A12: The size of plasmid affects the efficiency greatly. For instance, the efficiency for a supercoiled 2.7 Kb and a 10 Kb plasmid (using 1 minute protocol and FYE609) is 1.6~5.5 x 10⁹ and 4.0~9.0 x 10⁶, respectively. The difference is approximately 100 times. Transformation efficiency for large plsmids (especially for > 6 Kb) can be increased by using 6 min protocol as described in Q9.



Figure 4. The effect of plasmid sizes on transformation efficiency. The efficiency for 2.7 Kb and 10 Kb plasmid are 1.6~5.5 x10°/µg and 4.0~9.0 x 10°/µg, respectively.

Q13: How to perform blue/white screening?

A13: Please make sure the plasmid, which have been transformed to ECOS™, contains the *LacZ* operon. After transformation, please spread ECOS™ competent cells onto LB plates (containing 0.5 mM IPTG and 40~60 µg/mI X-gal). After incubation at 37°C, white colonies indicate insertion of foreign DNA on *LacZ* operon, and blue colonies indicate no insertion leading to functional β-galactosidase activity to hydrolyze the X-gal.

Q14: How to use the control plasmid (pUC19) in package?

A14: There is 5 µl of 10⁻⁴ µg/µl pUC19 in each package for positive control. Please dilute it to 10⁻⁶-10⁻⁷ µg/µl before transformation. Add 1 µl 10⁻⁶-10⁻⁷ µg/µl pUC19 to 1 vial ECOS™ competent cells and follow one of the ECOS™ transformation protocols. It is suitable for 20 µg/ml ampicillin selection only.

Q15: What are the differences in package for different E. coli strains?

A15: Each *E. coli* strain is color coded in the packing box and tube caps for easy distinguishment if they are mixed.

If kanamycin is used for selection and the plasmid size > 6 Kb, we suggest using an improved recovery protocol, in which 0.4~0.9 ml LB broth is added to the cells followed by shaking at 37 °C for 20~60 min before plating.

BG: Background (pseudo antibiotics resisitants)

	Ampicillin (μg/ml) Chloramphenicol (μg/ml)						Kanamycin (μg/ml) Tetracycline (μg/							
Product	Plasmid (size)	20	50	20	30		10	15	20	25	7.5	15	25	
ECOS™ Blue	pUC19 (2.7 Kb)	8.3x10 ⁸	1.1x10 ⁹											
(Cat. No. FYE109)	pUC4k (4.0 Kb)	1.0x10 ⁹	5.6x10 ⁸				2.0x10 ⁸	1.2x10 ⁸	7.2x10 ⁷	3.3x10 ⁷				
(strain: XL1-Blue)	pBR325-KR (7.4 Kb)	2.0x10 ⁸	2.2x10 ⁸	2.0x10 ⁸	1.5x10 ⁸		*	*	*	*				
ECOS™ 21	pUC19 (2.7 Kb)	1.3x10 ⁸	4.7×10 ⁷											
(Cat. No. FYE207) (strain: BL21(DE3)	pUC4k (4.0 Kb)	1.2x10 ⁸	3.2x10 ⁷				BG	BG	1.6x10 ⁷	6.0x10 ⁶				
	pBR325-KR (7.4 Kb)	8.0x10 ⁶	3.0x10 ⁵	2.0x10 ⁷	1.6x10 ⁷		BG	*	*	*	2.0x10 ⁷	1.8x10 ⁷	6.0x10 ⁵	
ECOS™ 101	pUC19 (2.7 Kb)	1.4x10 ⁹	9.2x10 ⁸											
(Cat. No. FYE609)	pUC4k (4.0 Kb)	9.6x10 ⁸	8.6x10 ⁸				BG	9.2x10 ⁷	3.5x10 ⁷	1.6x10 ⁷				
(strain: DH5α)	pBR325-KR (7.4 Kb)	3.0x10 ⁸	2.8x10 ⁸	4.0x10 ⁸	2.5x10 ⁸		BG	*	*	*	3.0x10 ⁸	1.0x10 ⁸	1.3x10 ⁷	
ECOS™ 9-5	pUC19 (2.7 Kb)	4.6x10 ⁸	3.5x10 ⁸											
(Cat. No. FYE708)	pUC4k (4.0 Kb)	3.3x10 ⁸	1.6x10 ⁸				5.4x10 ⁷	1.6x10 ⁷	1.1x10 ⁷	2.7x10 ⁶				
(strain: JM109)	pBR325-KR (7.4 Kb)	7.8x10 ⁷	5.6x10 ⁶	1.3x10 ⁸	1.2x10 ⁸		*	*	*	*	BG	1.1x10 ⁸	3.0x10 ⁷	

Table 2. The test of super ECOS™ 101 transformation efficiencies under various conditions

* If kanamycin is used for selection and the plasmid size > 6 Kb, we suggest using an improved recovery protocol, in which 0.4~0.9 ml LB broth is added to the cells followed by shaking at 37 °C for 30~60 mins before plating.

Note: Please refer to Fig. 1, Cold plate, 1 min = protocol(1); Cold plate, 6 mins = protocol(3); Warm plate, 1 min = protocol(2); Warm plate, 6 mins = protocol(4).

		pUC19 (2.7 Kb)					pUC4k (4.0 Kb)					pBR325-KR (7.4Kb)			
		Warm Cold			Warm		Cold		Warm		Cold				
Antibiotic	Protocol	1 min	6 min	1 min	6 min		1 min	6 min							
(concentration)		(2)	(4)	(1)	(3)		(2)	(4)	(1)	(3)	(2)	(4)	(1)	(3)	
Ampicillin	(20 μg/ml)	5.5x10 ⁸	5.6x10 ⁸	9.2x10 ⁸	1.4x10 ⁹		2.9x10 ⁸	4.2x10 ⁸	7.8x10 ⁸	9.6x10 ⁸	1.3x10 ⁸	1.2x10 ⁸	1.8x10 ⁸	3.0x10 ⁸	
	(50 μg/m l)	3.5x10 ⁸	3.9x10 ⁸	1.0x10 ⁹	1.1x10 ⁹		1.9x10 ⁸	4.6x10 ⁸	9.5x10 ⁸	9.0x10 ⁸	1.0x10 ⁸	7.0x10 ⁷	2.7x10 ⁸	3.1x10 ⁸	
Kan annuala	(15 μg/ml)						1.3x10 ⁸	1.4x10 ⁸	7.3x10 ⁷	1.0x10 ⁸	*	*	*	*	
Kanamycin	(25 μg/ml)						2.1x10 ⁷	2.1x10 ⁷	1.0x10 ⁷	1.6x10 ⁷	*	*	*	*	
T-1	(7.5 μg/ml)										8.0x10 ⁷	8.5x10 ⁷	1.1x10 ⁸	1.1x10 ⁸	
Tetracycline (15 μg/r	(15 µg/ml)										3.0x10 ⁶	4.4x10 ⁷	5.0x10 ⁶	1.0x10 ⁸	
Obligation	(20 µg/ml)										7.5x10 ⁷	9.7x10 ⁷	1.8x10 ⁸	2.4x10 ⁸	
Chloramphenicol	(30 μg/ml)										3.0x10 ⁶	7.6x10 ⁷	1.1x10 ⁷	1.3x10 ⁸	



