Yeastern Biotech Co., Ltd

Product Use Limitation & Warranty

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Ver. M0925

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ECOS™ Competent Cells

1 Minute *E. coli* Transformation
SOC Eliminated
Revolution

Global Patents: US 6,864,088 US 7,098,033 I 229696 GB 2383582 FR 2832727 DE 10,251,429...
Trade Mark: USA, Japan, Canada, Korea, Taiwan...

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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 minure 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

Throughout the shipping process, two electronic temperature recorders are included in the package to monitor the shipping temperature at all times.
The shipping temperature is maintained within at -65±5 °C.

Storage and Expiration

ECOS™ Competent Cells must be stored 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 several freeze-thaw cycles. The efficiency of ECOS™ Competent Cells is good for 1 year with proper storage.

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Items and Ordering Information

Cat. No.	Product Name	Compatible to (strain)	Efficiency (cfu/μg)	Quantity
FYE107-10VL	Economic ECOS™ blue	*E. coli* XL1-blue	> $1x10^8$	100 μl × 10 vials
FYE107-80VL				100 μl × 80 vials
FYE108-10VL	ECOS™ blue		> $5x10^8$	100 μl × 10 vials
FYE108-80VL				100 μl × 80 vials
FYE109-10VL	Super ECOS™ blue		> $2x10^9$	100 μl × 10 vials
FYE109-80VL				100 μl × 80 vials
FYE207-5VL	ECOS™ 21 (DE3)	*E. coli* BL21 (DE3)	> $5x10^7$	100 μl × 5 vials
FYE207-40VL				100 μl × 40 vials
FYE607-10VL	Economic ECOS™ 101	*E. coli* DH5α	> $1x10^8$	100 μl × 10 vials
FYE607-80VL				100 μl × 80 vials
FYE608-10VL	ECOS™ 101		> $3x10^8$	100 μl × 10 vials
FYE608-80VL				100 μl × 80 vials
FYE678-10VL			> $5x10^8$	100 μl × 10 vials
FYE678-80VL				100 μl × 80 vials
FYE609-10VL	Super ECOS™ 101		> $1x10^9$	100 μl × 10 vials
FYE609-80VL				100 μl × 80 vials

Cat. No.	Product Name	Compatible to (strain)	Efficiency (cfu/μg)	Quantity
FYE610-10VL	ECOS™ X	*E. coli* DH5α	>$5x10^9$	100 μl × 10 vials
FYE610-80VL				100 μl × 80 vials
FYE707-10VL	Economic ECOS™ 9-5	*E. coli* JM109	>$5x10^7$	100 μl × 10 vials
FYE707-80VL				100 μl × 80 vials
FYE708-10VL	ECOS™ 9-5		>$1x10^8$	100 μl × 10 vials
FYE708-80VL				100 μl × 80 vials
FYE709-10VL	Super ECOS™ 9-5		>$5x10^8$	100 μl × 10 vials
FYE709-80VL				100 μl × 80 vials
FYE508-10VL	ECOS™ 10B	*E. coli* DH10B	>$1x10^8$	100 μl × 10 vials
FYE508-80VL				100 μl × 80 vials
FYE807-10VL	Economic ECOS™ 2163	*E. coli GM2163*	>$1x10^7$	100 μl × 10 vials
FYE807-80VL				100 μl × 80 vials

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

96-well Products

Cat. No.	Product Name	Efficiency (cfu/μg)	Quantity
FYE107-96WL	Economic ECOS™ blue	>5x10^7	50 μl × 96 wells
FYE108-96WL	ECOS™ blue	>2x10^8	50 μl × 96 wells
FYE109-96WL	Super ECOS™ blue	>5x10^8	50 μl × 96 wells
FYE207-96WL	ECOS™ 21	>2x10^7	50 μl × 96 wells
FYE607-96WL	Economic ECOS™ 101	>1x10^8	50 μl × 96 wells
FYE608-96WL	ECOS™ 101	>3x10^8	50 μl × 96 wells
FYE609-96WL	Super ECOS™ 101	>1x10^9	50 μl × 96 wells
FYE707-96WL	Economic ECOS™ 9-5	>5x10^7	50 μl × 96 wells
FYE708-96WL	ECOS™ 9-5	>1x10^8	50 μl × 96 wells
FYE709-96WL	Super ECOS™ 9-5	>5x10^8	50 μl × 96 wells
FYE508-96WL	ECOS™ 10B	>1x10^8	50 μl × 96 wells
FYE807-96WL	Economic ECOS™ 2163	>5x10^7	50 μl × 96 wells

Genotypes and Applications

Product	Genotype	Application
ECOS™ Blue	*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 *lac* [F'*pro*AB *lacI*^{q}ZΔM15 Tn10 (Tet^r)].	Suitable for blue-white color screening and routine cloning application.
ECOS™ 21	F$^-$ *omp*T *hsd*S$_B$($r_B^-m_B^-$) *dcm gal* λ(DE3)	Appropriate host for recombinant protein expression using T7-based expression vectors.
ECOS™ 101	*end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*F)U169 Φ80 Δ(*lac*Z)M15 F$^-$	Suitable for cloning with large plasmids and cDNA library construction, and also allow blue-white colony selection.
ECOS™ 9-5	*e*14$^-$(*McrA*$^-$) *rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17(r_K^- m_K^+) *sup*E44 *rel*A1 Δ(*lac-pro*AB) [F' *tra*D36 *pro*AB *lacI*^{q}ZΔM15]	Appropriate for blue-white color and robotic screening. It is a fast growing strain, forming visible colonies within 8 ~ 10 hours.

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Product	Genotype	Application
ECOS™ 10B	F⁻ *end*A1 *rec*A1 *gal*E15 *gal*K16 *nup*G *rps*L Δ*lac*X74 Φ80*lac*ZΔM15 *ara*D139 Δ*(ara,leu)*7697 *mcr*A Δ*(mrr-hsd*RMS-*mcr*BC*)* 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 *leu*B6 *fhu*A31 *lac*Y1 *tsx78* *gln*V44 *gal*K2 *gal*T22 *mcr*A *dcm*-6 *his*G4 *rfb*D1 *rps*L136 *dam*13::Tn9 *xyl*A5 *mtl*-1 *thi*-1 *mcr*B1 *hsd*R2	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 *E. coli* and yeast transformation	100 g

1 Minute Transformation Protocol

Heat shock / cold plating

1. 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 and step 2 cannot be done immediately, keeping the competent cells on ice will generate better efficiency.)
2. Add DNA (pre-chilled on ice, volume should be ≤ 5% of competent cells) immediately. Vortex for 1 second or tap the tube with finger to mix well. (Optional step (3) : 2~6 minutes protocol): Keep the tubes on ice for 1~5 min will increase the efficiency a little bit. If many transformations are carried out at one time, this optional step will facilitate the process and make the overall efficiency more uniform.)
3. Heat shock the cells in a water bath at 42 °C for 15~45 seconds. (For optional heat shock duration, please refer to Q8 on page 13.)
4. Plate the cells using Plating Beads (Cat. No.: FYO001-100G) onto a pre-chilled (4 °C) and dried selective LB agar plate (LB+antibiotics).
5. Incubate the plates at 37 °C (8~16 hours for ECOS™ 9-5, 12~16 hours for others).

Non-heat shock / warm plating

1. Prewarm a selective LB agar plate at room temperature upto 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 and step 2 cannot be done immediately, keeping the competent cells on ice will generate better efficiency.)
3. Add DNA (pre-chilled on ice, volume should be ≤ 5% of competent cells) immediately Vortex for 1 second or tap the tube with finger to mix well. (Optional step (4) : 2~6 minutes protocol);

Keeping the tubes on ice for 1~5 min will slightly increase the efficiency. If many transformations are carried out at a time, this optional step will facilitate the process and make the overall efficiency more uniform.

3. Plate the cells using Plating Beads (Cat. No.: FYO001-100G) onto the pre-warmed and dried selective LB agar plate (LB+antibiotics).
4. Incubate the plates at 37°C (8~16 hours for ECOS™ 9-5, 12~16 hours for others).

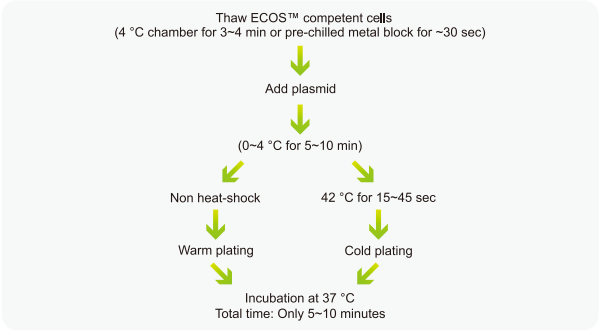
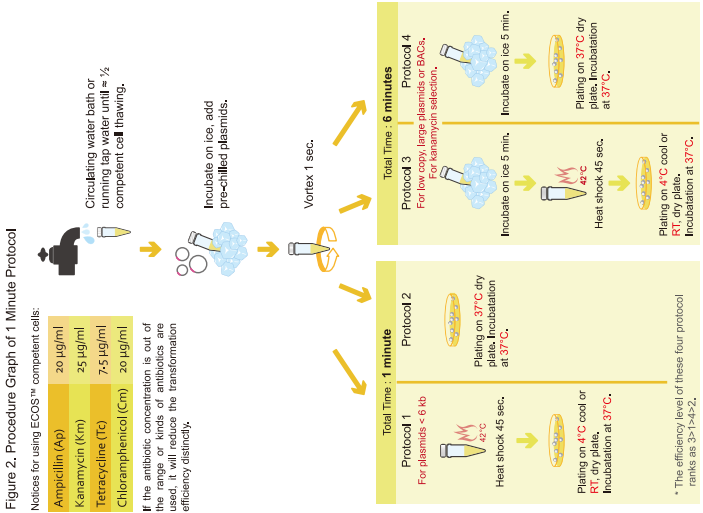


Figure 1. Automated / semi-automated 96 well ECOS™ Transformation Protocol



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Quality Control

1. Efficiency test: Each batch of ECOS™ is checked rigorously to meet the product specifications as described in pages 3-5 (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 μl of competent cells have been transformed with 10^{-6} μg of pUC19 plasmid. If 550 colonies are observed on the selective plate. The transformation efficiency is: $550/10^{-6} = 5.5 \times 10^{8}$ 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.

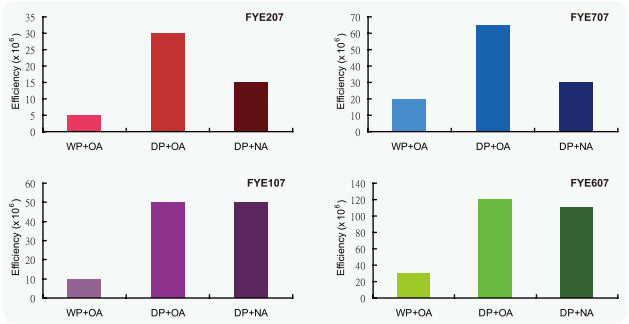

Figure 3. Effect of plate wetness and 50 μg/ml ampicillin on transformation efficiency for various *E. coli* strains (FYE107, FYE207, FYE607, FYE707) using 1 min protocol. The results showed that using dried plate with old batch of ampicillin have a higher transformation efficiency.

WP+OA: Wet plate with old batch (over 3 months from the date of opening) of ampicillin.
DP+OA: Dried plate with old batch of ampicillin.
DP+NA: 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 min protocol should be used to significantly improves the efficiency (Fig. 2, 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.

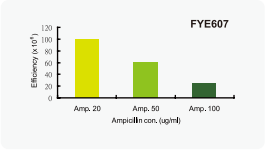

Figure 4. Effect of concentrations of ampicillin (fresh batch) on transformation efficiency using 1 min protocol with dried plate.
Amp. 20: 20 μg/ml ampicillin.
Amp. 50: 50 μg/ml ampicillin.
Amp. 100: 100 μg/ml ampicillin.

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Q12: Does the size of plasmid affect transformation efficiency?

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 10^9 and 4.0~9.0 x 10^6, 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.

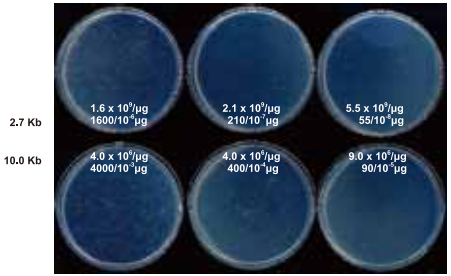

Figure 5. The effect of plasmid sizes on transformation efficiency. The efficiency for 2.7 Kb and 10 Kb plasmid are 1.6~5.5 x10^9/μg and 4.0~9.0 x 10^6/μg, respectively.

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/ml 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.

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Table 1. The efficiencies under various conditions including, strains, plasmid sizes, antibiotics concentrations by 6 min protocol (protocol 3).

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

✲ 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)

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Table 2. The test of super ECOS™ 101 transformation efficiencies under various conditions including, transformation method, plasmid sizes, and antibiotics concentrations.

		pUC19 (2.7 Kb)					pUC4k (4.0 Kb)				pBR325-KR (7.4Kb)			
		Warm		Cold			Warm		Cold		Warm		Cold	
Antibiotic (concentration)	Protocol	1 min	6 min	1 min	6 min		1 min	6 min	1 min	6 min	1 min	6 min	1 min	6 min
		(1)	(3)	(2)	(4)		(1)	(3)	(2)	(4)	(1)	(3)	(2)	(4)
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/ml)	$3.5x10^8$	$3.9x10^8$	$1.0x10^9$	$1.1x10^9$		$1.9x10^8$	$4.6x10^8$	$9.5x10^8$	$9.0x10^8$	$1.0x10^8$	$7.0x10^7$	$2.7x10^8$	$3.1x10^8$
Kanamycin	(15 μg/ml)						$1.3x10^8$	$1.4x10^8$	$7.3x10^7$	$1.0x10^8$	*	*	*	*
	(25 μg/ml)						$2.1x10^7$	$2.1x10^7$	$1.0x10^7$	$1.6x10^7$	*	*	*	*
Tetracycline	(7.5 μg/ml)										$8.0x10^7$	$8.5x10^7$	$1.1x10^8$	$1.1x10^8$
	(15 μg/ml)										$3.0x10^6$	$4.4x10^7$	$5.0x10^6$	$1.0x10^8$
Chloramphenicol	(20 μg/ml)										$7.5x10^7$	$9.7x10^7$	$1.8x10^8$	$2.4x10^8$
	(30 μg/ml)										$3.0x10^6$	$7.6x10^7$	$1.1x10^7$	$1.3x10^8$

✲ 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 min before plating.

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

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Xtremely Fast

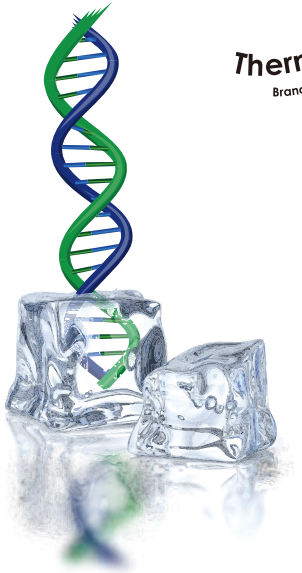
Xtremely Simple

Xtreme High Efficiency

NEW

Cat. #: FYE610-10VL
Cat. #: FYE610-80VL

ECOS™ X Competent Cells



Thermostable β-Agarase

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