Notices for using ECOS™ competent cells:

Ampicillin (Ap)	20 μg/ml
Kanamycin (Km)	25 μg/ml
Tetracycline (Tc)	7.5 μg/ml
Chloramphenicol (Cm)	20 μg/ml

If the antibiotic concentration is out of the range or kinds of antibiotics are used, there will reduce the transformation efficiency distinctly.

Circulating water bath or running tap water until ~1/2 competent cell thawing.

Incubate on ice, add pre-chilled plasmids.

Vortex 1 sec

Total time: 1 Minute

Protocol 1

For plasmids< 6Kb

Heat shock 45 sec

Plating on 4°C cool or RT, dry plate. Incubation at 37°C.

Protocol 2

Plating on 37°C dry plate. Incubation at 37°C.

Total time: 6 Minutes

Protocol 3

For low copy, large plasmids or BACs. For kanamycin selection.

Incubate on ice 5 min

Heat shock 45 sec

Plating on 4°C cool or RT, dry plate. Incubation at 37°C.

Protocol 4

Incubate on ice 5 min

Plating on 37°C dry plate. Incubation at 37°C.

* The efficiency level of these four protocol ranks as 3>1>4>2.

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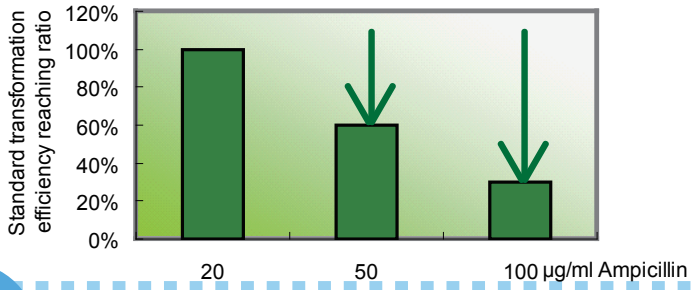
Please follow the suggestion for antibiotic selection concentration



If the concentration is out of the range, there will reduce the transformation efficiency distinctly.

Positive Control of ECOS™ Transformation

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.

Calculation of Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units produced by 1 μg plasmid DNA. For example, 100 μl of ECOS™ competent cells have been transformed with 10^{-6} μg pUC19. If 100 colonies are observed on the selective plate, the transformation efficiency is $100/10^{-6} = 1x10^{8}$ (cfu/μg).

Blue/white Screening

Please make sure the plasmids which have been transformed to ECOS™ contain the *Lac*Z operon. After transformation, please plating the ECOS™ competent cells to the LB plates (contain 0.5 mM IPTG and 40~60 μg/ml X-gal). After 37°C incubation, white colonies indicate insertion of foreign DNA on *Lac*Z operon and blue colonies still keep the functional β-galactosidase ability to hydrolyse the X-gal.

Composition of LB plate

10 g/L Tryptone
5 g/L Yeast extract
10 g/L NaCl
15 g/L Agar

Adjust the pH to 7.5 with NaOH.
Allow the medium cool down to 55°C after autoclave and add the antibiotic to suggested concentration (notices on other side).