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Manual

E.coli Transformer Express Kit

Kit for preparation of competent *E.coli* cells and further transformation. Improved chemical method that allows transformation even in 1 minute, without heat shocking.

cat#	size
4020-240E	6 x 40 transformations

For research use only.

Guarantee

A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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	4020-240E	storage
S1 Express solution	15 ml	+4 °C
S2 Express solution	15 ml	+4 °C

This kit was tested on derivatives of *Escherichia coli*: B, K-12 strains..

Additional equipment and reagents

Necessary for competent cells preparation

- *E.coli* strain
- sterile LB Miller medium (LB)
- sterile LB Agar medium (LA)
- sterile 1.5 ml Eppendorf tubes, sterile 50 ml Falcon tubes
- shaking incubator set to 37 °C
- centrifuge with cooling option, with rotor for 50 ml tubes

Necessary for competent cells transformation

- sterile SOC medium: 1 ml per transformation, cat # K-SOC-40, K-SOC-240
- selection medium plates: 1 plate for 1 transformation
- thermoblock set to 37 °C
- centrifuge with rotor for 1.5 ml tubes

Media preparation

Preparation of 1000 ml of medium.

LB Agar (LA)

1. Add **40 g** of medium to the appropriate vessel.
2. Add sterile water up to **1000 ml** and mix.
3. Autoclave for **10-20 min** at **121 °C**.
4. After cooling to **50-60 °C** mix again before use.

   Note. At 25 °C pH should be 7.0.

LB Miller (LB)

1. Add **25 g** of medium to the appropriate vessel.
2. Add sterile water up to **1000 m**l and mix.
3. Autoclave for **10-20 min** at **121 °C**.
4. After cooling to **50-60 °C** mix again before use.

   Note. At 25 °C pH should be 7.0.

Competent cells preparation protocol

- *Escherichia coli* should be streaked onto **LA** medium to obtain single colonies.
- Incubate plates overnight at **37 °C**.
- **S1 Express** and **S2 Express** must be cooled on ice.

1. Inoculate a single colony of *E.coli* obtained from LA plate into **10 ml** of **LB medium** (if necessary with appropriate antibiotic).
   Incubate **overnigh**t at **37 °C**.
2. Add **1 ml** of **overnight culture** to **100 ml** of fresh **LB medium** (if necessary with appropriate antibiotic).
3. Incubate in a shaking incubator for **2-3 hours** at **220-250 RPM** at **37 °C** until OD_{600}=0.3-0.5 (logarithmic growth phase).

   Attention. For some *E. coli* strains, incubation at **18 °C - 25°C** allows better competence.
4. Keep the culture **on ice** for **15 min**.
5. Centrifuge for **5 min** at **3500 RPM** (~1500 x g) at **+4 °C**. Discard the supernatant.

   Attention. If supernatant is not clear, increase centrifugation up to 3000 x g, 10 min.
6. Resuspend the pellet in **2 ml** of **S1 Express solution**.
7. Carefully add **2 ml** of **S2 Express solution** and gently mix.
8. Keep **on ice** for **15 min**.
9. Transfer **100 µl** of **competent cell suspension** to 1.5 ml tubes.

   Attention. 100 µl of competent cells should be used for one transformation. Avoid multiple freeze-thaw cycles.
10. Competent cells are ready for transformation or can be stored at -80 °C for later use.

    Attention. It is very important that competent cells are slowly frozen. Cells must not be frozen in liquid nitrogen.

Competent cells transformation protocol without heat shocking (1 - 5 minutes)

- Prepare plates with a selective medium.
- Before transformation, the medium should be at room temp.
- Heat the **SOC medium** to **37 °C**.
- It is recommended to prepare an additional plate with a selective medium for the negative control.

1. Use **100 µl** of ***E.coli* competent cells** for each transformation. Cells should be thawed on ice or freshly prepared and cooled on ice.

2. Add **plasmid DNA** or **ligation mixture** to the competent cells and gently mix.

   Attention. The volume of DNA / ligation mixture should not exceed 20 µl.

3. Keep **on ice** for **1-5 min**.

   Attention. During incubation avoid shaking or moving the tube. This reduces the effectiveness of the transformation. Longer incubation time improves the efficiency of the transformation.

4. If transformed plasmid is ampicillin resistant, plate the transformation mixture on LA medium with ampicillin.

5. If transformed plasmid is not ampicillin resistant (or with resistance for another antibiotic):
   Add **1 ml** of **SOC medium without antibiotics, preheated to 37 °C**.
   Incubate for **45 min** with shaking of **220 RPM** at **37 °C** to express genes responsible for antibiotic resistance.

6. Centrifuge for **3 min** at **3500 RPM** and remove most of the supernatant . Gently resuspend bacterial pellet in remaining supernatant and plate on LA medium with appropriate antibiotic.

7. Incubate **overnight** at **37 °C**.

Additional information

Average efficiency of transformation of *E.coli* TOP10F' cells with pUC19 plasmid is 10^7-10^8 CFU/µg. To increase the efficiency of the transformation, e.g. in the case of complicated cloning, we recommend the following changes to the protocol:

- increasing the incubation time on ice to **45 min.** (follow point 3. of the competent cell transformation protocol)
- then heat shocking **60 s** at **42 °C**
- keep **on ice** for **2 min**
- follow point 4. of the competent cell transformation protocol.

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