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Manual

E.coli Transformer Kit

Kit for preparation of competent *E.coli* cells and transformation. Chemical method.

cat#	size
4020-240	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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Contents

	4020-240	storage
S1E solution	15 ml	+4 °C
S2E 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) (cat.# 2020-250, 2020-1000)
- sterile LB Agar medium (LA) (cat.# 2021-250, 2021-1000)
- sterile 1.5 ml Eppendorf tubes, sterile 50 ml Falcon tubes
- shaking incubator set to 37 °C
- centrifuge with rotor for 50 ml tubes

Necessary for competent cells transformation

- sterile LB Miller medium (LB) (cat.# 2020-250, 2020-1000)
- selection medium plates: 1 plate for 1 transformation
- sterile 50 ml Falcon tubes
- thermoblock set to 37 °C
- centrifuge with rotor for 50 ml tubes

Media preparation

Preparation of 1000 ml of medium.

LB Agar (LA) (cat.# 2021-250, 2021-1000)

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) (cat.# 2020-250, 2020-1000)

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 reductively onto LA medium
- Incubate plates overnight at 37 °C
- S1E and S2E solutions must be cooled on ice

1. Inoculate a single colony of *E.coli* obtained from reduction culture 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).

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.

   If supernatant is not clear, increase centrifugation up to 3000 x g up to 10 min.

6. Resuspend the pellet in **2 ml** of **S1E solution**.

7. Carefully add **2 ml** of **S2E solution** and gently mix.

8. Keep **on ice** for **15 min**.

9. Transfer **100 µl** of **competent cell suspension** to 1.5 ml tubes.

   100 µl of competent cells should be used for one transformation. Avoid multiple freeze-thaw cycles.

10. Competent cells are ready for transformation (page 5.) or can be stored at -80 °C for later use.

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

Competent cells transformation protocol

- Prepare plates with a selective medium.
- Before transformation, the medium should be at room temp.
- 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.

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

3. Keep **on ice** for **15-45 min**.

   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. Perform a heat shocking **60 s** at **42 °C**.

   It is also possible to perform this step in an ultrasonic bath. Mixture should be placed in an activated ultrasonic bath for 5-10 s at room temp.

5. Keep **on ice** for **2 min**.

   If transformed plasmid is ampicillin resistant follow point 7.

6. If transformed plasmid is not ampicillin resistant (or with resistance for another antibiotic):
   - add **1 ml** of **preheated to 37 °C LB media without antibiotics**.
   - incubate for **45 min** with shaking at **220 RPM** at **37 °C** to express genes responsible for antibiotic resistance.

7. Cultivate **20-200 µl** of **transformation mixture** on a plate with a selective medium.

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

Average efficiency of transformation of *E.coli* TOP10F' cells with pUC19 plasmid is 10^7-10^8 CFU.

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