TransformAid™ Bacterial Transformation Kit

#K2710
Lot: ___ Exp.: ___
Quantity: for 20 transformations

#K2711
Lot: ___ Exp.: ___
Quantity: for 40 transformations

PRECAUTION
Wear gloves to avoid contact with skin

IMPORTANT!
Store at -20°C.
Repeated freeze-thawing diminishes the quality.

COMPONENTS* OF THE KIT

1. **C-Medium:**
   35ml (70ml) of ready-to-use solution.
2. **T-Solution (A):**
   2.5ml (5ml) (2(4)x1.25ml) of ready-to-use solution.
3. **T-Solution (B):**
   2.5ml (5ml) (2(4)x1.25ml) of ready-to-use solution.

* *Do not contain glucose.*

STORAGE

The T-Solution (A) and T-Solution (B) should be stored at -20°C. The C-Medium should be stored at -20°C (in case of long -term storage) or at 4°C. For convenience, store the working C-Medium at 4°C.

PROTOCOL

Preparation of Bacteria from Overnight Culture

1. Inoculate 2ml of TransformAid™ C-Medium with bacteria from a frozen stock or a colony. Incubate the culture at 37°C overnight.

2. Pre-warm culture tubes containing the required amount of TransformAid™ C-Medium (1.5ml for each of 2 transformations) at 37°C.

3. Add 1/10 volume of the overnight culture to the pre-warmed C-Medium (0.15ml overnight culture for each 1.5ml C-Medium). Incubate the tubes in a shaker at 37°C for 20min.

Note

A maximum of 26 transformations can be performed from 2ml of overnight culture. The culture can be kept at 4°C for at least a week and used for preparation of fresh cultures.

Preparation of Bacteria from Bacterial Culture Plate

1. Inoculate a LB plate with bacteria using streak plate method, and incubate the plate at 37°C overnight.

2. Pre-warm culture tubes containing the required amount of TransformAid™ C-Medium (1.5ml for each 2 transformations) at 37°C.

3. Move a small portion of bacterial culture (4 x 4mm size for each 1.5ml of C-Medium) from the overnight LB plate using inoculating loop in to the pre-warmed C-Medium. Suspend the culture by gently mixing and incubate the tubes in a shaker at 37°C for 2 hours.

Note

The colonies on LB plates can be stored at 4°C and used for inoculating fresh cultures within 10 days.

Transformation Procedure

1. Pre-warm LB-Ampicillin agar plates (*see* Note 1) in a 37°C incubator for at least 20min.

2. Prepare TransformAid™ T-Solution by mixing equal volumes of T-Solution (A) and T-Solution (B) (500μl of solution for each 2 transformations). Keep TransformAid™ T-Solution on ice (*see* Note 2).

3. Dispense 1.5ml of fresh culture into a microcentrifuge tube and spin at maximum speed for 1min at room temperature (RT) or at 4°C (*see* Note 3).

4. Discard the supernatant and resuspend the pelleted cells in 300μl of TransformAid™ T-Solution. Incubate the tubes on ice for 5min.

5. Spin down the cells again for 1min at RT or at 4°C and then remove the supernatant.

6. Resuspend the cells in 120μl of TransformAid™ T-Solution and incubate on ice for 5min.

7. Prepare DNA for transformation by dispensing 1μl of supercoiled DNA (10-100pg) or up to 5μl of ligation mixture (10-100ng of vector DNA) into new microcentrifuge tubes and sit them on ice for 2min (*see* Note 4).

8. Add 50μl of the resuspended cells to each tube containing DNA and incubate them on ice for 5min.

9. Plate the cells on pre-warmed LB-Ampicillin agar plates. Incubate the plates overnight at 37°C.

Note

- DNA containing other antibiotic resistance genes can be used in the TransformAid™ system. The transformation efficiency should first be tested with supercoiled DNA containing these antibiotic genes. In our hands, DNA containing tetracycline and chloramphenicol resistance genes have similar transformation efficiency as DNA containing the ampicillin resistance gene (~10^6 to 10^7). However, DNA containing the kanamycin resistance gene has a lower transformation efficiency (~10^5).

- After thawing **T-Solution (A)** and **T-Solution (B)**, mix contents thoroughly prior to combining equal volumes of T-Solution (A) and T-Solution (B).

- The centrifugation can be carried out at room temperature (RT), but the cells should be kept on ice at all other times as indicated. Do not leave the cells in the centrifuge at RT for more than 5min as this will significantly decrease the transformation efficiency.

- The quantity of DNA used in transformation will influence the transformation efficiency. The transformation efficiency usually decreases with an excess of DNA. In the TransformAid™ system, approximately 100 to 2000 colonies will be obtained using 100pg of supercoiled DNA for most bacterial strains (DH5alfa®, JM107, JM109, SURE®, TOPP2®, W3110, NM527, AD494 and CJ236). To transform cells with ligation reaction mixture, a reaction volume not larger than 5μl (containing 10-100ng of vector DNA) should be used. We usually obtain 50-200 colonies for simple sticky end ligation reactions.

Scale-up

When preparing a large volume of competent cells for transformation, the transformation procedure can be scaled up proportionately. The cells can be pelleted in large centrifuge tubes at 5000-10,000xg for 5min in steps **3** and **5** from transformation procedure.

Plaque Formation

To obtain plaques, use 10ng double-stranded phage DNA (e.g. **M13mp19**) to transform cells. Follow the usual transformation procedure for **steps 1-8**. In step **9**, mix the transformed cells (from step **8**) with 4ml top agar (0.7% at 55°C). After gentle mixing, pour the agar/cell mixture onto an agar plate. The number of plaques obtained depends on the bacterial strain used (CJ236: ~ 60 plaques, JM109: ~ 350 plaques).

Preparation of LB-Ampicillin Plates

1. Prepare LB Medium (per liter):

Peptone	10g
Yeast extract	5g
NaCl	10g

2. Dissolve solids in 800ml of water, adjust pH to 7.5 with NaOH.

3. Adjust water to a final volume of 1000ml.

4. Autoclave the medium.

Prepare LB-Ampicillin plates:

5. Adjust 15g agar to 1 liter of LB medium.

6. Microwave the medium until the agar dissolves.

7. Allow the solution to cool down to 55°C, then add ampicillin to a final concentration of 50μg/ml.

   (For convenience, prepare a stock solution of ampicillin at 25mg/ml. Use 2ml of the stock solution per liter of LB-agar medium. Store the stock solution at -20°C.)

8. Mix gently and pour 30-35ml of LB-agar medium directly onto each plate.

For other antibiotics, use the following concentrations:

- Kanamycin, 30μg/ml
- Tetracycline, 12μg/ml
- Chloramphenicol, 20μg/ml.

Note

The concentration of antibiotic in the agar plates will affect the number of colonies obtained. The antibiotic resistance of transformants is dependent on the copy number of the plasmid used. When a low copy number plasmid is used, the concentration of the antibiotic can be decreased in order to obtain a greater number of transformants.

Ligation Reaction Protocol

DNA Insert Ligation into Vector DNA

1. In a microcentrifuge tube prepare 5-10μl mix in water or TE buffer of digested (if preferred dephosphorylated and purified) vector DNA (50-400ng) and foreign DNA to be inserted.

2. Add the following components to the same tube:

10X ligation buffer	2μl
50% PEG 4000 solution (for blunt ends only)	2μl
deionized water up to	20μl
T4 DNA ligase	1-2u (for sticky ends), 5u (for blunt ends)

   Vortex the tube and spin down in a microcentrifuge for 3-5sec.

3. Incubate the mixture for 1 hour at 22°C.

4. Inactivate T4 DNA ligase by heating reaction mixture at 65°C for 10 minutes.

5. Use the mixture for transformation.

Note

1. TE buffer 10mM Tris-HCl, 1mM EDTA (pH 7.8).
2. Use equal or higher (up to 3-fold) molar concentration of insert DNA termini over vector DNA.
3. If the yield of ligation product is insufficient, prolong the reaction time (overnight).
4. The resulting ligation reaction mixture can be used directly for bacterial transformation. An excess of ligation mixture in regard to competent cells may decrease the transformation efficiency.

Self-circularization of Linear DNA

1. In a microcentrifuge tube prepare a solution of linear DNA (25-50ng) in deionized water or TE buffer (10-35μl).

2. Add the following components to the same tube:

10X ligation buffer	5μl
50% PEG 4000 solution (for blunt ends only)	5μl
deionized water up to	50μl
T4 DNA ligase	5u

3. Vortex the tube and spin down in a microcentrifuge for 3-5sec. Incubate the tube for 1 hour at 22°C.

4. Inactivate T4 DNA ligase by heating reaction mixture at 65°C for 10 minutes.

5. Resulting reaction mixture can be used directly for transformation.

QUALITY CONTROL

All components of the Kit are tested for transformation efficiency using pUC19 DNA and bacterial strains XL1-Blue and JM107.

MSDS

Product Use Limitation