

Product information

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Single Step Ultra-Competent Cell Preparation Kit

Catalog #: BS523 | BS524
Size: 25ml | 50ml
Storage: (-15 to -20)°C

Description:

SSCS is supplied as a ready to use format of 1x solution.

Application: Preparation of E. coli competent cells for transformation.

This Kit contains SSCS solution, enables researchers to prepare competent E. coli cells in a single step and to transform the cells without heat-shock. SSCS has been reported to be faster and easier than other methods of producing competent cells, such as the traditional CaCl₂ method described by Sambrook et al, or other high competency protocols. The competent cells obtained by this method can be kept at -70°C for long term storage. Transformation efficiencies depend on the strain of E. coli, as well as the nature and quality of the transforming DNA. Typical transformation efficiencies are 10⁶ to 10⁸ pfu/μg plasmid DNA. For example, the transformation efficiencies observed for E. coli strains DH1, DH5a, HB101, Jm109, Le392, MM294, SCS-1, XL1-blue and TG1 ranged from 1.5×10⁶ to 1.0×10⁸ pfu per μg of DNA.

Contents:

Component	BS523	BS524
SSCS Solution	25 ml	50 ml
Protocol	1	1

Features:

- Simple & Fast. Entire procedure takes about 5 minutes.
- Stable at -70°C with little or no loss in transformation efficiency. It is suitable for long term storage.
- No heat shock required.
- High transformation efficiencies of 10⁶ to 10⁸ pfu/μg DNA are typically obtained.
- Reproducible.

Quality Control:

Transformation and storage solution is tested for transformation efficiency with appropriate E. coli strains and pUC18 or pUC19 DNA.

Storage:

All contents of the kit should be kept in freezer after usage. The Kit is stable for 9 months.

Procedure (Single Preparation):

1. Pick a colony from an overnight cultured agar plate containing the appropriate strain of E. coli using a sterile tooth pick or a sterile pipette tip. Inoculate the colony into 2 ml SOB broth and grow overnight on a shaker at 37°C with vigorous shaking.
2. On Day 2, inoculate 1 ml of the above culture into 50 ml of SOB broth in a 250 ml flask. Continue to grow the cells on the shaker at 37°C with vigorous shaking until $OD_{600}=0.5\sim0.7$.
-  3. Transfer 1 ml supernatant into a clean tube. Spin at 2,000 x g (4000 rpm) for 2~3 minutes and discard the supernatant carefully.
-  4. Add 100 µl of per-cold SSCS solution and re-suspend the cells gently.
-  5. Add 100 pg to 10 ng of transforming DNA to the cells, mix gently and incubate the mixture on ice for 10 minutes, then 37°C for 5 minutes, followed by on ice for 10 minutes.
-  6. Add 1 ml pre-warmed SOB broth and incubate the tube at 37°C for 45-60 min.
7. Plate the cells on the appropriate selective or differential media.

Procedure (Batch Preparation):

1. Pick a colony from an overnight cultured agar plate containing the appropriate strain of E. coli using a sterile tooth pick or a sterile pipette tip. Inoculate the colony into 2 ml SOB broth and grow overnight on a shaker at 37°C with vigorous shaking.
2. On Day 2, inoculate 1 ml of the above culture into 50 ml of SOB broth in a 250 ml flask. Continue to grow the cells on the shaker at 37°C with vigorous shaking until $OD_{600}=0.5\sim0.7$.
-  3. Transfer culture to a 50 ml centrifuge tube. Spin at 2,000 x g (4000 rpm) for 2~3 minutes and discard the supernatant carefully.
-  4. Add 5 ml of per-cold SSCS solution and re-suspend the cells gently.
-  5. Aliquot the cell mixture 50~100 µl per tube. The competent cells are ready to use or can be stored at -70°C.
-  6. Perform E. coli transformation according to standard protocol.

Notes:

1. For large volume of cell preparation, the volume of SSCS should be increased correspondingly. For example, 1~2 ml SSCS solution can be used for 50~100 ml cultured cells. After the cells are resuspended, aliquot of 50~100 µl should be dispensed and then stored at -70°C.
2. Steps 3 to 6 **MUST BE CARRIED OUT ON ICE AT ALL TIMES.**
3. The solution works well with *Bacillus subtilis*.



PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.
NOT INTENDED FOR HUMAN OR ANIMAL USE.