A&A BIOTECHNOLOGY
innovating life science

Manual

JO-FI™ competent cells

High efficiency competent cells suitable for any type of cloning method and standard plasmid DNA transformation. Stable replication of high-copy plasmids.

catalog #	size
1033-20	20 pcs
1033-40	40 pcs

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee 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

Advantages

- High transformation efficiency: 5-6 x 10^9 cfu/µg pUC19 DNA

Description

JO-FI™ competent cells are derived from the *E. coli* DH5α. The strain is not pathogenic and has been developed specifically for molecular cloning (Biohazard level 1).

Specification

- Tetracycline (15 µg/ml) and Streptomycin (25 µg/ml) resistance.
- The $lacI^q$ repressor enables tight, IPTG -dependent expression control from the *trc*, *tac* and *lac* promoters.
- The lacZΔM15 deletion allows the white-blue color selection of recombinant clones (LA + X-Gal).
- *recA1* gene mutation reduces the occurrence of undesired recombination events during cloning.
- *endA1* gene mutation decreases endonuclease I activity. This results in a lower level of DNA degradation, providing faster plasmid DNA transfer.
- *mcrA* gene mutation allows efficient transformation of methylated genomic DNA.
- *hsdR* gene mutation allows efficient transformation of unmethylated DNA after PCR amplification.

Contents

	1033-20	**1033-40**	**storage**
JO-FI™ competent cells	20 x 50 µl	40 x 50 µl	-80 °C
pUC19 control DNA (10 pg/µl)	50 µl	50 µl	from -80 up to -20 °C
SOC medium	20 ml	40 ml	-20 °C

Good practices during transformation

Thawing

Thaw cells on ice. Mild thawing conditions ensure best results. Add DNA immediately after the last piece of ice in the tube disappears.

Incubation on ice

To reach maximum transformation efficiency, cells and DNA should be incubated on ice for a minimum 30 min. Shorter incubation reduces transformation efficiency.

Heat shock

Both temperature and heat shock time affects the transfer of plasmid DNA. Do not exceed parameters specified in the protocol.

Growing transformants

Growing of the transformants culture at 37 °C for 60 min is optimal for cell recovery and expression of antibiotic resistance. Decreasing the incubation time below 45 min reduces the transformation efficiency. SOC medium leads to increased transformation efficiency in relation to the standard LB medium.

Transformation plates

Both warm or cold plates, wet or dry, can be used for transformation without significantly affecting the performance. However, it is easier to distribute the ligation mixture on warm and dried plates, and the formation of bacterial colonies can be observed sooner.

Transformation protocol

1. Thaw cells completely on ice (about **10 min**).
2. Add **1-5 µl** (1 pg-100 ng) of plasmid **DNA**.
3. Gently mix by pipetting.

   Attention! Do not vortex.
4. Incubate on ice for **30-60 min**.
5. Incubate for **30-60 s** at **42 °C**.
6. Incubate on ice for **5 min**.
7. Add **900 µl** of **SOC** medium (pre-warmed to room temperature) without antibiotics.
8. Incubate in a shaker for **45-60 min** at **37 °C**.
9. Centrifuge for **2 min** at **2 000-3 000 RPM**.
10. Remove **800 µl** of supernatant. Mix the pellet by pipetting.
11. Transfer **100 µl** of suspension to a LA plate with the appropriate antibiotic and spread over the entire surface.
12. Incubate plates for:
    16 h at **37 °C** or
    24-35 h at **30 °C** or
    48 h at **25 °C**

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