SmartCellsTM High Efficiency Chemically Competent *E. coli*

Cat. #	Contents	Quantity
AMS.C101020	SmartCells™ Chemically Competent E. coli	20 x 50 µl
	(>1 x 10 ⁹ cfu/ µg)	
	SOC Medium	6 ml
	pUC19 Positive Control Plasmid	20 μl (10pg/ μl)
AMS.C101120	SmartCells™ F' Chemically Competent E. coli	20 x 50 µl
	(>1 x 10 ⁹ cfu/µg)	
	SOC Medium	6 ml
	pUC19 Positive Control Plasmid	20 µl (10pg/ µl)

Shipping	Shipped on Dry Ice
Storage	Store cells at -70°C; store the SOC Medium at room
	temperature. Stable for 6 months.

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Related Products	Catalog #
EZ-Spread ™ Beads, Single-Use Tubes, 50 tubes	AMS.C400050
EZ-Spread [™] Beads, Dispenser Bottle	AMS.C400100

Introduction: Most commercially available chemically competent cells, regardless of claimed efficiency, frequently underperform in real transformation experiments, especially when using ligation mixtures instead of supercoiled DNA. SmartCellsTM chemically competent *E. coli* are prepared by a unique procedure to warrant the highest and most robust transformation performance under diverse conditions. There is no need to dilute or purify your ligation mix before transformation. If needed, over 10 μ l of full strength ligation mix can be added to 50 μ l competent cells without significantly compromising transformation results. SmartCellsTM carry genotypes suitable for most cloning needs such as blue/white selection, generation of plasmid vector based libraries or gene banks, and ability to be transformed efficiently with large plasmids. SmartCellsTM F' is also available for use with M13 cloning vectors.

SmartCellsTM F⁻: recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ 80lacZ Δ M15 Δ (lacZYA-argF)U169 SmartCellsTM F⁻: recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 ϕ 80lacZ Δ M15 Δ (lacZYA-argF)U169

METHODS AND PROCEDURES

A. Transformation Protocol

- 1. Thaw one tube of SmartCells[™] on ice (10-15 minutes).
- 2. Transfer thawed cells to a chilled 2059 Falcon tube.
- 3. Add 1-10 μl of ligation mix to the cells. Mix gently and incubate on ice for 15 to 30 minutes.

NOTE: In our test, up to 10 μ l of undiluted ligation mix could be added without compromising transformation efficiency.

- 4. Heat the mix at 42°C for 45 seconds.
- 5. Immediately place the transformation tube on wet ice for two minutes.
- 6. Add 0.95 ml room temperature SOC medium and incubate at 37°C for 1.5 hours in an air incubator with shaking (225 rpm).
- 7. Dilute transformation reaction if necessary and spread 100 µl of transformed cells on LB/Agar plates containing appropriate selection (e.g. ampicillin or kanamycin). Alternatively, if maximum numbers of colonies are desired, collect cells by spinning in a microfuge for 10 seconds. Resuspend cell pellet in 50 µl SOC and spread on the agar plates containing antibiotics.

B. Positive Control Reaction

To perform a positive control reaction using the provided supercoiled pUC19 plasmid DNA, perform the following steps:

- 1. Repeat steps 2-6, but add 5 μl (50 pg) pUC19 to 50 μl of competent cells in Step 3.
- Dilute the transformation reaction 50 fold by adding 10 μl of it to 490 μl SOC; plate 100 μl of this dilution on an LB agar plate containing 100 μg/ml carbenicillin or ampicillin.
- Incubate overnight at 37°C and count colonies. Calculate transformation efficiency as follows:

 $\frac{\text{Number of Colonies}}{50 \text{ pg pUC19}} x \frac{1 \text{ x } 10^6 \text{ pg } \text{ x } 500 \text{ µl}}{100 \text{ µl plated}} x 50^{\dagger} = \text{CFU/µg}$

[†] 50 is the dilution factor

8. Incubate overnight at 37°C.

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