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Product Information

JM109 Competent Cells

Catalog Number **J3895**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

JM109 competent *Escherichia coli* is an all purpose cloning strain that is ideal for the generation of high quality plasmid DNA.[1] JM109 is a K strain bacterium that carries the *rec*A1 and *end*A1 mutations. The *rec*A1 genotype provides minimized recombination and aids in plasmid stability while *end*A1 provides for high quality plasmid DNA preparation. The strain carries the *hsd*R17 genotype, which prevents cleavage of heterologous DNA by an endogenous endonuclease. JM109 cells also contain an F' episome carrying Δ(*lacZ*)M15 for blue/white screening via α-complementation with the amino terminus of β-galactosidase. The presence of the F' factor also allows growth of bacteriophage M13 vectors for rescue of single stranded DNA.

Sigma's JM109 competent *E. coli* cells are grown and made chemically competent using an optimized procedure specific to the strain, followed by strain verification and efficiency testing. The cells are provided in frozen 50 µL aliquots for convenience. Each aliquot may be used for a single transformation. The cells are guaranteed to have a transformation efficiency of $>1x10^8$ cfu/µg when transformed with non-saturating amounts of pUC19 plasmid DNA.

Genotype of JM109:

F′ (traD36, *pro*AB+ *lac*I^q, Δ(*lac*Z)M15) *end*A1 *rec*A1 *hsd*R17(r_k^-, m_k^+) *mcr*A *sup*E44 λ^- *gyr*A96 *rel*A1 Δ(*lac-proAB*) *thi*-1

Reagents

JM109 Competent Cells are packaged with sufficient reagents for 10 transformation reactions.

- JM109 Competent Cells, 10 x 50 µL, Catalog No. J4020
- pUC19 Control DNA, 10 ng/µL, 10 µL, Catalog No. D2567

Reagents and Equipment Required but Not Provided

(Sigma Product Numbers have been given where appropriate)

- Shaker incubator (37 °C)
- Cabinet incubator (37 °C)
- Heated water bath (37 °C)
- SOC Medium, Catalog No. S1797
- LB Agar EZMix™, Catalog No. L7533
- Appropriate selection antibiotic
- 15 ml polypropylene culture tubes (sterile)
- Culture dishes
- Sterile Spreaders, Catalog No. Z376779
- IPTG (Isopropyl-β-D-thiogalactoside), Catalog No. I6758
- X-gal, Catalog No. B9146
- S-Gal™, Catalog No. S9811
- Ferric Ammonium Citrate, Catalog No. F5879

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

All components are stable for at least six months after receipt when properly stored at –70 °C.

Procedure

Handling Tips:

1. Verify that the cells are still frozen and dry ice is still present in the shipping container upon receipt.
2. Handle the tubes as little as possible to prevent accidental warming of the cells. Keep cells on ice at all times.
3. When mixing the cells, gently swirl or tap the reaction tube. Do not mix by pipetting or vortexing.
4. Cells can be refrozen on dry ice and returned to –70 °C, however transformation efficiency will decrease significantly with each freeze-thaw cycle.

Before Starting:

1. Prepare LB agar plates with the appropriate antibiotic for selection of colonies that contain plasmid DNA, and warm to 37 °C. If blue/white screening for recombinants is desired, the plates should include 1 mM IPTG and 300 µg/ml S-Gal or 40 µg/ml X-gal + 500 µg/ml Ferric Ammonium Citrate.
2. Heat a water bath to 37 °C.

Note: Alternatively, the water bath may be set to 42 °C, but this variation on the procedure has been shown to be slightly less robust than 37 °C.

3. Warm SOC medium to room temperature (20-25 °C).

I. Standard Transformation Protocol In Brief

Note: See following section for detailed protocol

1. Thaw the required tubes of cells on wet ice.
2. Add 1–50 ng of DNA (or 1 µL of control DNA) to cells and gently tap the tube to mix.
3. Place cells on wet ice for 30 minutes.
4. Heat tubes for **exactly** 45 seconds in a 37 °C water bath.
5. Return the cells to ice for 2 minutes.
6. Add 450 µL of SOC, at room temperature, to each transformation reaction.
7. Shake at 225 rpm at 37 °C for 1 hour.
8. Use a sterile spreader to evenly distribute transformation reaction over LB agar plates containing the appropriate selection antibiotic.
9. Incubate plates overnight at 37 °C.

II. Detailed Standard Transformation Protocol

Note: DNA in ligation reactions containing high quality reagents is suitable for direct addition to Sigma's competent cells. Inactivation of the ligase is not required prior to transformation.

Plasmid DNA isolated using miniprep procedures is typically satisfactory. To achieve maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein, and detergents, and be dissolved in TE buffer or water.

1. Remove the required number of tubes from the –70 °C freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice so that only the cap is visible above the ice. Allow the cells to thaw on ice for approximately 5 minutes.
2. Visually examine cells to ensure they are thawed, and gently tap the vial several times to resuspend cells.
3. (Optional) Add 1 µL pUC19 Control DNA (10 ng) to one tube of cells. Mix gently by tapping the tube. Return the cells to the ice.
4. Add 1 ng to 50 ng ligation reaction or purified plasmid DNA directly to cells. Mix as in Step 3.
5. Incubate the cells on wet ice for 30 minutes.
6. Incubate the cells in a 37 °C water bath for **exactly** 45 seconds.
7. Return the cells to ice immediately for 2 minutes.

8. Add 450 µL of SOC medium, at room temperature, into each tube containing the cell/DNA mixture. Optimal recovery may be achieved by transferring the cells to a sterile 15 ml polypropylene culture tube. The cap should be loose to ensure sufficient air exchange and aeration of the culture.

9. Incubate cells at 37 °C with shaking (225-250 rpm) for 1 hour.

10. Pipette 10-100 µL of each transformed cell suspension onto LB agar plates containing selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal transformation efficiency. When inoculating less than 25 µl of cell suspension, first pipette a drop of SOC onto the plate and add cell suspension to the SOC.

Note: The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. When using the control DNA, plate no more than 10 µL into a drop of SOC on an LB agar plate containing 100 µg/ml freshly made ampicillin or carbenicillin.

11. Incubate plates overnight at 37 °C.

12. Select a colony and culture as needed.

III. Rapid Transformation Procedure

Note: Rapid transformation may be used with high quality plasmid DNA. Although considerable time can be saved (up to 60 minutes) following the rapid transformation protocol, a loss of 10- to 100-fold transformation efficiency is expected.

1. Follow the previous Handling Tips and Before Starting tips, page 2.

2. When using Kanamycin for antibiotic selection, the standard transformation protocol is recommended.

3. Remove the required number of tubes from the –70 °C freezer, including one extra for the control DNA if desired. Place tubes immediately on wet ice, so that only the cap is visible above the ice. Allow the cells to thaw for approximately 5 minutes.

4. Visually examine cells to ensure they are thawed, and flick the vial several times to resuspend cells.

5. (Optional) Add 1 µL pUC19 Control DNA (10 ng) to one tube. Mix gently by tapping the tube. Return the tube to the ice.

6. Add 1-50 ng of ligation reaction to the cells. Mix as in Step 3 and incubate on ice 3-5 minutes.

7. Heat shock the cells by incubating in a 37 °C water bath for **exactly** 45 seconds.

8. Immediately return the tubes to ice and incubate for 2 min.

9. Add 200 µL of SOC, at room temperature, to the cells.

10. Pipette 20-200 µL of each transformed cell suspension onto LB agar plates containing the selection antibiotic and evenly distribute using a sterile spreader. Plates should be pre-warmed to 37 °C for optimal transformation efficiency. When inoculating with less than 25 µL of cells, first pipette a drop of SOC onto the plate and add cell suspension to the SOC.

Note: The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. When using the control DNA, add no more than 10 µL of the cell suspension to a drop of SOC on an LB agar plate containing 100 µg/ml freshly made ampicillin or carbenicillin

11. Incubate overnight at 37 °C

References

1. Yanisch-Perron, C., *et al*., Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, **33,** 103-119 (1985).

Troubleshooting Guide

Problem	Cause	Solution
No Colonies on plate	Cells thawed and re-frozen due to improper handling	Obtain new product, Temperature of ultracold freezer should be –70 °C or colder
	Old or expired cells	Obtain new product
	No plasmid DNA added	Ensure plasmid DNA was added to cells
	Input plasmid DNA missing antibiotic resistance ORF and/or replication of origin	Review cloning strategy
	Low efficiency ligation	Plate increased volume of transformation reaction
	Too much, or wrong antibiotic used	Adjust antibiotic levels in plates, Test a plate by streaking with an antibiotic resistant culture
	Low efficiency transformation	Check to make sure transformation protocol was followed exactly
		Plate increased volume of transformation reaction
		Check efficiency using included pUC19 control
Too Many Colonies on Plate or a Lawn of Growth is Observed.	High efficiency transformation or high amounts of input DNA	Plate decreased volume of transformation reaction
	Too little antibiotic	Adjust antibiotic levels in plates
	Expired antibiotic	Use fresh antibiotic in plates
	Contaminated SOC media	Obtain fresh medium and maintain sterile technique
Unexpected Growth on Plate (i.e. fungal or mold)	Contaminated plates	Prepare and spread plates in laminar flow hood to reduce contamination
	Contaminated SOC media	Obtain fresh medium and maintain sterile technique

Please visit www.sigma-aldrich.com for a complete listing of competent cells and other transformation products offered by Sigma-Aldrich.

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