



MaV203 Competent Yeast Cells, Library Scale

Cat. No. 11281-011

Size: 1.1 ml

Store at -80°C

(Do not store in liquid nitrogen)

Description

MaV203 Competent Yeast Cells have been developed for use with the ProQuest™ Two Hybrid System to facilitate library-scale transformations. One transformation using 250 µl of these highly competent cells will yield $\geq 1 \times 10^6$ colonies. MaV203 contains deletions in the endogenous *GAL4* and *GAL80* genes for use with most GAL4-based two-hybrid systems. This strain has been constructed with three GAL4-inducible reporter genes for identification of interacting fusion proteins: *GAL1::lacZ*, *HIS3_{UASGAL1}::HIS3* and the counterselectable *SPAL10::URA3* (1-3). MaV203 is also auxotrophic for leucine (*leu2*) and tryptophan (*trp1-901*), allowing for the selection of yeast transformed with *GAL4* DNA binding domain (DB) vectors and *GAL4* activation domain (AD) vectors (e.g., pDBLeu and pPC86 from the ProQuest™ Two-Hybrid System). MaV203 also contains the recessive resistance alleles *cyh2^R* and *can^R* which are useful for plasmid shuffling.

Component	Amount per Vial
MaV203 Competent Yeast Cells	2 × 550 µl
PEG/Lithium Acetate (LiAc) Solution	5 × 1.5 ml
pMAB12 DNA, 0.25 µg/µl	10 µl
pMAB37 DNA, 0.25 µg/µl	10 µl

Genotype

MATα; *leu2-3,112*; *trp1-901*; *his3A200*; *ade2-101*; *cyh2^R*; *can1^R*; *gal4A*; *gal80A*; *GAL1::lacZ*; *HIS3_{UASGAL1}::HIS3@LYS2*; *SPAL10::URA3*.

Note: While the genotype of MaV203 is *ade2*, the strain is phenotypically *ADE2⁺*. The genetic basis of this phenotypic reversion is under investigation.

Control Plasmids

The control DNAs pMAB37 (*TRP1*) and pMAB12 (*LEU2*) provided with this kit are used to confirm the transformation efficiency of the cells and that the selection plates were prepared correctly. These plasmids do not contain the elements necessary to do a screen with the ProQuest™ Two-Hybrid System.

Part No. 11281011

Doc. Rev. 121202

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Transformation Procedure

Library Scale Transformation Procedure: 500 μ l of cells are expected to generate $\geq 2 \times 10^6$ colonies (enough for one library screen). **Note:** The optimum concentration of 3AT for DNA binding domain vectors containing test gene X **must** be determined prior to doing a library transformation.

1. Thaw the PEG/LiAc Solution in a beaker containing room temperature water before the assay. Mix the solution well before dispensing.
2. Thaw competent cells by placing in a 30°C water bath for 90 seconds. Do not allow the cells to remain at 30°C longer than 90 seconds. Proceed immediately to Step 3. Steps 3, 4, and 5 can be done at room temperature.
3. Once the cells are completely thawed, invert the cells several times. Transfer 250 μ l to two 15 or 50 ml polypropylene tubes (see Note 1). **Do not vortex the cells.** Transfer 25 μ l of cells to two 1.5 ml microcentrifuge tubes and simultaneously perform the control assay (see next page).
4. To each 250 μ l aliquot of cells, add 10 μ g (≥ 0.2 μ g/ μ l) of DNA binding domain vector containing a test gene and the 10 μ g (≥ 0.2 μ g/ μ l) of activation domain vector containing a library to be screened. Mix well by swirling the tubes. **Note:** The total volume of DNA added to the transformation reaction must be no more than 100 μ l.
5. Add 1.5 ml of the PEG/LiAc Solution to each tube. Mix well by swirling the tubes until all of the components are homogeneous.
6. Incubate for 30 minutes in a 30°C water bath. Swirl the tubes occasionally (every 10 minutes) to resuspend the components.
7. Add 88 μ l of DMSO to each tube (see Note 2). Mix well by swirling the tube.
8. Heat shock the cells for 20 minutes in a 42°C water bath. Swirl the tubes occasionally.
9. Centrifuge each tube for 5 minutes at 1800 rpm (640 \times g) in a tabletop centrifuge. Carefully discard the supernatant.
10. Suspend each pellet in 8 ml of autoclaved saline (0.9% NaCl). Combine the suspensions into one tube.
11. Remove 100 μ l and dilute 1:100 and 1:1000 in autoclaved saline. Plate 100 μ l of each dilution on 10 cm SC-Leu-Trp plates. Incubate plates at 30°C for 60-72 hours.
12. For the remaining mixture, plate 400 μ l aliquots onto forty 15 cm SC-Leu-Trp-His+3AT plates.
13. Incubate the plates for 3 days at 30°C. For use with the ProQuest™ Two-Hybrid System, follow the replica cleaning procedures outlined in the manual.

Control Transformation Procedure: This assay should be done side by side with the library transformation.

1. Add 4 μ l of pMAB37 DNA and 4 μ l of pMAB12 DNA to one 25 μ l aliquot of cells and mix well by swirling. Do not add DNA to the second 25 μ l aliquot.
2. Add 180 μ l PEG/LiAc Solution to the tubes and mix well by inversion. Be sure the components are homogeneous.
3. Incubate for 30 minutes in a 30°C water bath. Mix occasionally.
4. Add 10.8 μ l of DMSO and mix well by inversion.
5. Heat shock for 20 minutes in a 42°C water bath. Swirl the tubes occasionally.
6. Centrifuge for 5 seconds at low speed (1800 rpm; 200-400 \times g) in a microcentrifuge. Carefully remove the supernatant and discard.
7. Suspend each pellet of cells in 1 ml of autoclaved saline by gentle pipetting.
8. Dilute the cells 1:100 and 1:1000 in autoclaved saline. Plate 100 μ l of each dilution on 10 cm SC-Leu-Trp plates.
9. Incubate plates at 30°C for 72 hours. The expected number of colonies per reaction is $\geq 5 \times 10^4$ (see Note 3).

Notes

1. **Do not freeze thaw.** Competent yeast can only be thawed once without dramatic loss in competency.
2. For best results, use fresh DMSO from an unopened bottle. DMSO which has been stored at -20°C also works well.
3. Number of colonies per transformation reaction = Colonies/plate \times dilution factor.
4. For example, if 75 colonies are counted when 100 μ l of a 1:100 dilution are plated, the calculation would be:

$$75 \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10^2 = 7.5 \times 10^4 \text{ colonies/reaction}$$

References

1. Vidal, M., Brachmann, R.K., Fattaey, A., Harlow, E., and Boeke, J.D. (1996) *Proc.Natl. Acad. Sci.* 93, 10315.
2. Vidal, M., Braun, P., Chen, E., Boeke, J.D., and Harlow, E. (1996) *Proc.Natl. Acad. Sci.* 93, 10321.
3. Vidal, M. (1997) The Reverse Two-Hybrid System in *The Two-Hybrid System* (Barel, P. and Fields, S., eds.), Oxford University Press, New York, 109.
4. Hill, J. K., Donald, A.I.G., and Griffiths, D.E. (1991) *Nucleic Acids Res.* 19, 5791.

Quality Control

MaV203 Competent Yeast Cells will consistently yield $\geq 1 \times 10^6$ cotransformants with 250 μ l cells and saturating amounts of pDBLeu and pPC86 DNAs (10 μ g each).

Information for European Customers

The MaV203 yeast strain is genetically modified and contains three GAL4-inducible reporter genes stably integrated into the genome. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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