MaV203 Competent Yeast Cells, Library Scale

Catalog 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. A single transformation using 250 µL of these highly competent cells yields ≥1 × 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; his3Δ200; ade2-101; cyh2^R*; can1*^{R}*; gal4Δ; gal80Δ; GAL1::lacZ; HIS3*$_{UASGAL1}$*::HIS3@LYS2; SPAL10::URA3.*

Note: While the genotype of MaV203 is *ade2*$^-$, the strain remains white upon starvation for adenine or amino acids, but retains an *ade*2 deficiency.

Control Plasmids

The control DNAs pMAB37 (*TRP*1) and pMAB12 (*LEU*2) 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.

Library Scale Transformation Procedure

500 µL of cells are expected to generate ≥2 × 10^6 colonies (enough for one library screen). **Note:** You **must** determine the optimum concentration of 3AT for DNA binding domain vectors containing test gene X prior to 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. After the cells are completely thawed, invert the cells several times. Transfer 250 µL to two 15- or 50-mL polypropylene tubes (see Note 1). **Do not vortex the cells**. Transfer 25 µL of cells to two 1.5-mL microcentrifuge tubes and simultaneously perform the control assay (see next page).
4. To each 250 µL aliquot of cells, add 10 µg (≥0.2 µg/µL) of DNA binding domain vector containing a test gene and the 10 µg (≥0.2 µg/µL) 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 not exceed 100 µL.
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 µL of DMSO to each tube (see Note 2). Swirl the tube to mix.
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 (200–400 × *g*).
10. Carefully discard the supernatant and reuspend each pellet in 8 mL of autoclaved saline (0.9% NaCl). Combine the resuspensions into one tube.
11. Remove 100 µL and dilute 1:100 and 1:1000 in autoclaved saline. Plate 100 µL 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 µL 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

1. Add 4 µL of pMAB37 DNA and 4 µL of pMAB12 DNA to one 25 µL aliquot of cells and mix well by swirling. Do not add DNA to the second 25 µL aliquot.
2. Add 180 µL 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 µL 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 minutes at low speed (1800 rpm; 200–400 × *g*). Carefully remove the supernatant and discard.
7. Carefully discard the supernatant and reuspend each pellet in 1 mL of autoclaved saline by gentle pipetting.
8. Dilute the cells 1:100 and 1:1,000 in autoclaved saline. Plate 100 µL 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 × dilution factor. For example, if 75 colonies are counted when 100 µL 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.

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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