invitrogen

M13K07 Helper Phage

Catalog no. 18311-019 Size: 5 × 1 ml
Store at 4°C

Description

M13K07 Helper Phage is an M13 bacteriophage containing the origin of replication from p15A and the kanamycin resistance gene from Tn903 (1). M13K07 Helper Phage is used to generate highly purified ssDNA from F´ episome containing cells (such as DH11S™ Competent Cells, Invitrogen Cat. no. 18307-017) that have been transformed with phagemid vectors (2). M13K07 Helper Phage is prepared by a modification of the procedure of Vieira and Messing (1).

Component	Part No.	Amount per Vial
M13K07 Helper Phage	Y00166	1 ml

Quality Control

Titer of M13K07 Helper Phage: $\geq 1 \times 10^{11}$ pfu/ml

Growth of F′ Cells Containing Phagemid Vectors for Preparation of ssDNA

1. Pick a single colony of a transformed F´ host containing a phagemid vector with a sterile toothpick.
2. Resuspend the colony in 2 ml TBG (1.2% tryptone, 2.4% yeast extract, 0.4% glycerol, 17 mM KH_2PO_4, 55 mM K_2HPO_4, and 20 mM glucose) containing 100 µg/ml ampicillin in a 15 ml tube.
3. Add 10 µl of M13K07 Helper Phage.
4. Incubate the culture at 37°C with shaking at 275 rpm for 2 hours.
5. Add kanamycin to a final concentration of 75 µg/ml and incubate culture at 37°C, with shaking at 275 rpm for 18-24 hours. (See Note 3, next page).

Protocol continued on next page

Growth of F′ Cells Containing Phagemid Vectors for Preparation of ssDNA, continued

6. Transfer 1.5 ml of culture to a sterile microcentrifuge tube and pellet the cells at 14,000 rpm at 4°C for 10 minutes.
7. Transfer the supernatant to a new microcentrifuge tube and spin at 14,000 rpm at 4°C for 10 minutes.
8. In a new microcentrifuge tube, add 300 µl 2.5 M NaCl in 40% PEG 4000 to 1.2 ml of the supernatant.
9. Vortex and incubate on ice for 15 minutes.
10. Centrifuge at 14,000 rpm at 4°C for 15 minutes.
11. The pellets may be either:

    A.) Resuspended in 50 µl 1X TE buffer, extracted twice with phenol, precipitated with ethanol, and resuspended in 50 µl 1X TE **or**

    B.) Resuspended in 50 µl 1X TE with 100 µg Proteinase K and 0.1% SDS and incubated at 42°C for 1 hour.
12. For gel electrophoresis, 10 µl of sample is sufficient.

Notes

1. M13K07 Helper Phage is stable at either 4°C or –80°C. You may freeze/thaw up to five times without significant loss of titer.
2. For the best yields of ssDNA, M13K07 Helper Phage MOI must be >1 when cells are in stationary phase, and >10 when cells are in the log phase.
3. Addition of kanamycin during incubation increases the yield of ssDNA, however it may be omitted if small amounts of ssDNA are needed.

References

1. Vieira, J. and Messing, J., (1987) *Methods in Enzymol. 153*, 3.
2. Lin, J., Smith, M., Jessee, J., and Bloom, F., (1991) *FOCUS® 13:3*.