M13KO7 Helper Phage

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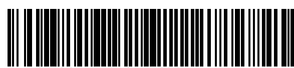

N0315S 020130115011

N0315S

1.8 ml **Lot: 0201301** **Exp: 1/15**

1.0 x 10^{11} pfu/ml **Store at –20°C**

Description: M13KO7 is an M13 derivative which carries the mutation Met40Ile in gII, with the origin of replication from P15A and the kanamycin resistance gene from Tn903 both inserted within the M13 origin of replication (1). M13KO7 is able to replicate in the absence of phagemid DNA. In the presence of a phagemid bearing a wild-type M13 or f1 origin, single-stranded phagemid is packaged preferentially and secreted into the culture medium.This allows easy production of single-stranded phagemid DNA for mutagenesis or sequencing.

Source: M13KO7 phage supernatant was isolated from infected *E. coli* ER2738 by a standard procedure (2).

Supplied in: 1X PBS and 50% glycerol.

Quality Control Assays

Absolute titer: Infection of a mid-log culture of *E. coli* ER2690 followed by plating yielded 1.0 x 10^{11} pfu/ml.

Viable cell titer: Plating the phage suspension for viable cells on LB agar yielded a titer of < 10^2/ml.

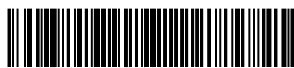
Helper ratio: Superinfection of an early-log culture of *E. coli* ER2738/pLITMUS 38 with 3 x 10^8 pfu/ml M13KO7, followed by incubation for 18 hours in the presence of 70 µg /ml kanamycin, yielded a supernatant that was tested for relative titers of packaged helper phage vs. phagemid as follows: The supernatant was heated at 65°C to kill any viable cells. A mid-log culture of *E. coli* ER2690 was briefly incubated with diluted supernatant and then plated both for plaques (to determine M13KO7 titer) and colonies on ampicillin plates (to determine packaged pLITMUS 38 titer). The ratio of ampicillin-resistant colonies to plaques was 10:1. Agarose gel electrophoresis confirmed a 10-fold excess of packaged phagemid DNA over helper phage.

Procedure for isolation of single-stranded phagemid DNA:

1. Transform phagemid vector into appropriate F´ strain (CJ236 for Kunkel mutagenesis).
2. Inoculate 50 ml LB (no antibiotic) with a fresh colony, grow at 37°C with vigorous aeration until slightly turbid (<10 Klett, A_{600} < 0.05). For Kunkel mutagenesis, add uridine to 0.25 µg/ml.
3. Add 50 µl M13KO7 Helper Phage (final concentration of 1 x 10^8 pfu/ml), continue vigorous aeration for 60–90 minutes.
4. Add kanamycin to final concentration of 70 µg/ml, grow overnight (14–18 hours) with vigorous aeration.
5. Spin culture at 8,000 rpm for 10 minutes. Transfer supernatant to a new tube and spin again.
6. Pipet the upper 90% of supernatant into a new tube. To this supernatant, add a 0.2 volume of 2.5 M NaCl/20% PEG. Incubate at 4°C for 60 minutes.
7. Recover the phage by centrifugation at 8,000 rpm for 10 minutes. Decant supernatant and spin again briefly.
8. Resuspend the pellet in 1.6 ml TE, transfer to 2 microfuge tubes.
9. Spin in a microfuge for 1 minute to pellet any remaining cells, transfer supernatant to new tubes.
10. Add 200 µl of the 2.5 M NaCl/20% PEG solution to each, let sit at room temperature for 5 minutes, spin in a microfuge for 10 minutes.
11. Decant the supernatant, spin again briefly, remove last traces of supernatant with pipetman.
12. Resuspend each pellet in 300 µl TE. Extract with phenol (let sit 15 minutes before spinning), then phenol/chloroform (50/50: v/v; twice), and finally chloroform. Add 30 µl 2.5 M NaOAc, pH 4.8 and alcohol precipitate.

(See other side)

CERTIFICATE OF ANALYSIS



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13. Suspend the dried pellets in 25–50 µl TE. Yield should be >50 µg single-stranded phagemid for pUC origin vectors.

NEB does not recommend the use of M13KO7 as a cloning vector. For cloning phage display libraries, we recommend the Ph.D. Cloning System.

References:

1. Vieira, J. and Messing, J. (1987) *Methods Enzymol.* 153, 3–11.
2. Sambrook, J. and Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual*, (3rd ed.), (pp. 3.17–3.32). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

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