M13KE Phage

1-800-632-7799
info@neb.com
www.neb.com

N0316S 00715091709 1

N0316S

0.04 ml **Lot: 0071509** **Exp: 9/17**

1.0 x 10^{13} pfu/ml **Store at –20°C**

Description: M13KE Phage is a suspension of infectious virions derived from the Ph.D. cloning vector, M13KE (1). The vector phage may be a useful control for phage ELISA or for titering of phage stocks. For cloning, the isolated replicative form of M13KE is available as part of the Ph.D.™ Peptide Display Cloning System (NEB #E8101S).

Source: M13KE phage was isolated from infected *E. coli* ER2738 by a standard procedure (2).

Supplied in: 1X TBS and 50% glycerol.

Quality Control Assays

Absolute titer: Infection of a mid-log culture of *E. coli* ER2738 followed by plating yielded 1.0 x 10^{13} pfu/ml.

Sequence verification: Sequencing of M13KE was carried out with –96 gIII Sequencing Primer (20-mer) (NEB #S1259S). Single digests of M13KE vector by KpnI and EagI were carried out to confirm presence of cloning sites.

Wild-type M13 contamination: A plate with >10^3 blue plaques showed no white plaques.

Protocol:

M13 Amplification

1. Grow overnight culture of F´+ *E. coli* (e.g. ER2738).
2. Inoculate a 20 ml culture in a 250 ml Erlenmyer flask with 200 µl overnight *E. coli* culture. Add 1 µl phage suspension. Shake flask at 37°C, 250 rpm for 4 –5 hrs.
3. Remove cells by centrifugation at 4500 g for 10 min. Transfer supernatant to a fresh tube. Repeat centrifugation.
4. Transfer top 16 ml of supernatant to a new tube and add 4 ml of 2.5 M NaCl/20 % PEG-8000 (w/v). Briefly mix. Precipitate phage for 1 hr or overnight at 4°C.
5. Pellet phage by centrifugation at 12000 g for 15 min. Decant supernatant. Resuspend pellet in 1 ml TBS. Transfer to an eppendorf tube. Spin briefly to remove any cell debris.
6. Transfer supernatant to a fresh tube. Add 200 µl of 2.5 M NaCl/20 % PEG-8000. Incubate on ice for 15-60 min. Spin 12000 –14000 rpm in a benchtop centrifuge for 10 min. Discard supernatant. Spin again briefly and remove remaining supernatant with pipette. Resuspend pellet in 200 µl TBS. For long-term storage at –20°C, add 200 µl sterile glycerol.

To scale up the above protocol, use multiple culture flasks. Alternatively, after incubating 20 ml culture for 2 hrs, add the entire culture to 1L LB. Incubate the large culture for 4 hrs, then modify the protocol to remove cells and purify phage.

Companion Products Sold Separately:

Ph.D. Peptide Display Cloning System
#E8101S 20 µg

Ph.D.-7 Phage Display Peptide Library Kit
#E8100S 10 panning experiments

Ph.D.-12 Phage Display Peptide Library Kit
#E8110S 10 panning experiments

Ph.D.-C7C Phage Display Peptide Library Kit
#E8120S 10 panning experiments

References:

1. Noren, K. A. and Noren, C. J. (2001) *Methods* 23, 169-178.
2. Sambrook, J. and Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual,* (3rd ed.), (pp3.17-3.32). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

CERTIFICATE OF ANALYSIS

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ISO 14001 Registered Environmental Management
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CERTIFICATE OF ANALYSIS