M13KE Phage

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

N0316S 006120314031

N0316S

0.04 ml **Lot: 0061203** **Exp: 3/14**

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

M13KE Phage

N0316S 006120314031

N0316S

0.04 ml Lot: 0061203 Exp: 3/14

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