

## M13KE Phage



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N0316S 007161018101

# N0316S

**0.04 ml**      **Lot: 0071610**      **Exp: 10/18**  
**1.0 x 10<sup>13</sup> 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<sup>13</sup> 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<sup>8</sup> 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 Erlenmeyer 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.



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

CERTIFICATE OF ANALYSIS

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