

Ph.D.[™]-12
Phage Display
Peptide Library



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www.neb.com



E8111L 013140216021

E8111L

0.5 ml **Lot: 0131402** **Exp: 2/16**
1.0 x 10¹³ pfu/ml **Store at -20°C**

Description: The Ph.D.[™]-12 Phage Display Peptide Library is based on a combinatorial library of random dodecapeptides fused to a minor coat protein (pIII) of M13 phage (1–6). The displayed peptide (12-mer) is expressed at the N-terminus of pIII, i.e., the first residue of the mature protein is the first randomized position. The peptide is followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. The library consists of approximately 10⁹ electroporated sequences amplified once to yield approximately 100 copies of each sequence in 10 µl of the supplied phage.

Supplied in: TBS with 50% glycerol.

Complexity: 1.7 x 10⁹ pfu.

Quality Control Assays

Control Panning Experiment: Approximately 10¹¹ phage (10 µl) is diluted with 100 µl TBST and is exposed to streptavidin as a target (see The Ph.D.-12 Phage Display Peptide Library Kit Manual). To complex any biotin in the BSA, the blocking reagent is prepared by adding 0.1 µg/ml streptavidin to the standard blocking solution. The bound phage is eluted with 0.1 mM biotin in TBS for at least 30 minutes. After 3 rounds of enrichment/amplification, the consensus sequence for streptavidin-binding peptides was determined to contain the motif: H P Q (6).

Epitope mapping with the Ph.D.-12 Library:

The library was panned against anti-β-endorphin, and anti-Flag monoclonal antibodies in solution, followed by affinity capture of the antibody-phage complexes onto Protein A-agarose (rounds 1 and 3) or Protein G-agarose (round 2). The results clearly show the epitopes for these antibodies, YGGF, and DYKXXD, respectively.

Deep sequencing was carried out with Ion Torrent™ technology on the naïve library:

Amino Acid	Codons	Expected frequency*	Observed frequency
Arg	CGK, AGG	9.4%	5.7%
Leu	CTK, TTG	9.4%	8.9%
Ser	TCK, AGT	9.4%	11.2%
Ala	GCK	6.2%	7.4%
Gly	GGK	6.2%	5.8%
Pro	CCK	6.2%	8.1%
Thr	ACK	6.2%	7.8%
Gln	CAG, TAG‡	6.2%	3.9%
Val	GTK	6.2%	6.1%
Asn	AAT	3.1%	4.5%
Asp	GAT	3.1%	4.6%
Cys	TGT	3.1%	1.5%†
Glu	GAG	3.1%	3.1%
His	CAT	3.1%	4.6%
Ile	ATT	3.1%	3.4%
Lys	AAG	3.1%	2.3%
Met	ATG	3.1%	3.1%
Phe	TTT	3.1%	2.7%
Trp	TGG	3.1%	2.3%
Tyr	TAT	3.1%	3.6%

* Expected frequency = # codons for that amino acid ÷ 32 codons x 100%. Note use of reduced genetic code NNK (32 codons) in library construction.

† Arginines and single cysteines in the random peptide sequence interfere with secretion of pIII and phage infectivity, respectively; consequently, clones with peptides containing Arg or Cys are selected against.

‡ The stop codon TAG is suppressed by Gln in the strain used to propagate the library.

(See other side)

CERTIFICATE OF ANALYSIS

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References:

1. Parmley, S.F. and Smith, G.P. (1988) *Gene* 73, 305–318.
2. Smith, G.P. and Scott, J.K. (1993) *Methods Enzymol.* 217, 228–257.
3. Reviewed in Cortese et al. (1995) *Curr. Opin. Biotechnol.* 6, 73–80.
4. Scott, J.K. and Smith, G.P. (1990) *Science* 249, 386–390.
5. Cwirla, S.E., Peters, E.A., Barrett, R.W. and Dower, W.J. (1990) *Proc. Natl. Acad. Sci. USA* 87, 6378–6382.
6. Devlin, J.J., Panganiban, L.C. and Devlin, P.E. (1990) *Science* 249, 404–406.

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References:

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