Ph.D.™-12 Phage Display Peptide Library

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

E8111L 0131408 16081

E8111L

0.5 ml **Lot: 0131408 Exp: 8/16**

1.0 x 10^{13} 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 10^9 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 10^9 pfu.

Quality Control Assays

Control Panning Experiment: Approximately 10^{11} 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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Cys	TGT	3.1%	1.5%†
Glu	GAG	3.1%	3.1%
His	CAT	3.1 %	4.6%
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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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