Ph.D.™-C7C Phage Display Peptide Library

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

E8121L 008140216021

E8121L

0.5 ml **Lot: 0081402** **Exp: 2/16**

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

Description: The Ph.D.-C7C Phage Display Peptide Library is based on a combinatorial library of random heptapeptides fused to a minor coat protein (pIII) of M13 phage (1–6). Unlike other Phage Display Libraries from NEB, the randomized sequence is flanked by a pair of cysteine residues. Under nonreducing conditions the cysteines will spontaneously form a disulfide cross-link, resulting in phage display of cyclized peptides, in contrast to the linear peptides displayed in the Ph.D.-7 and Ph.D.-12 libraries. Disulfide-constrained peptide libraries (7) have proven useful in identification of structural epitopes (8,9), mirror-image ligands for D-amino acid targets (10) and leads for peptide-based therapeutics (11). The disulfide-constrained heptapeptides are expressed at the N-terminus of pIII, with the first cysteine preceded by an alanine residue and the second cysteine followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. The library consist of 10^9 electroporated sequences amplified once to yield approximately 200 copies of each sequence in 10 µl of the supplied phage.

Supplied in: TBS with 50% glycerol.

Complexity: 2.7 x 10^9 transformants.

Quality Control Assays

Control Panning Experiment: Approximately 2 x 10^{11} phage (10 µl) is diluted with 100 µl TBST and is exposed to streptavidin as a target (see The Ph.D. -C7C 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: C G X $^{F}/_{Y}/_{W}$ X H P $^{Q}/_{M}$ C (6).

Amino Acid Distribution of the Ph.D.-C7C Library:

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%	5.59% †
Leu	CTK, TTG	9.4%	8.53%
Ser	TCK, AGT	9.4%	10.44%
Ala	GCK	6.2%	5.28%
Gly	GGK	6.2%	4.22%
Pro	CCK	6.2%	6.97%
Thr	ACK	6.2%	9.45%
Gln	CAG, TAG‡	6.2%	5.31%
Val	GTK	6.2%	4.18%
Asn	AAT	3.1%	6.75%
Asp	GAT	3.1%	3.95%
Cys	TGT	3.1%	0.59%†
Glu	GAG	3.1%	3.45%
His	CAT	3.1 %	4.95%
Ile	ATT	3.1%	3.16%
Lys	AAG	3.1%	5.13%
Met	ATG	3.1%	4.27%
Phe	TTT	3.1%	1.94%
Trp	TGG	3.1%	2.18%
Tyr	TAT	3.1%	3.64%

*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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2. Smith, G.P. and Scott, J.K. (1993) *Methods Enzymol.* 217, 228–257.
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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.
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10. Schumacher, T.N. M, Mayr, L.M. Minor, D.L. Milhollen, M.A., Burgess, M.W. and Kim, P.S. (1996) *Science* 271, 1854–1857.
11. Wrighton, N.C. et al. (1996) *Science* 271, 458–463.

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