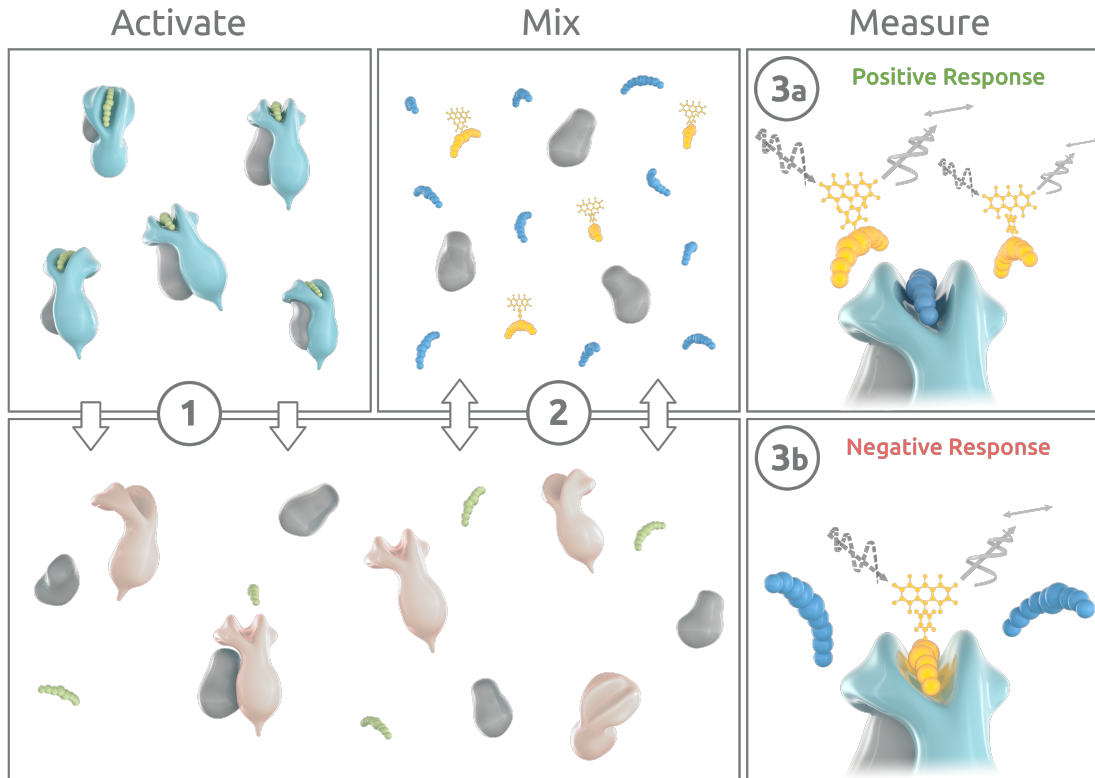


Peptide Epitope Screening Report



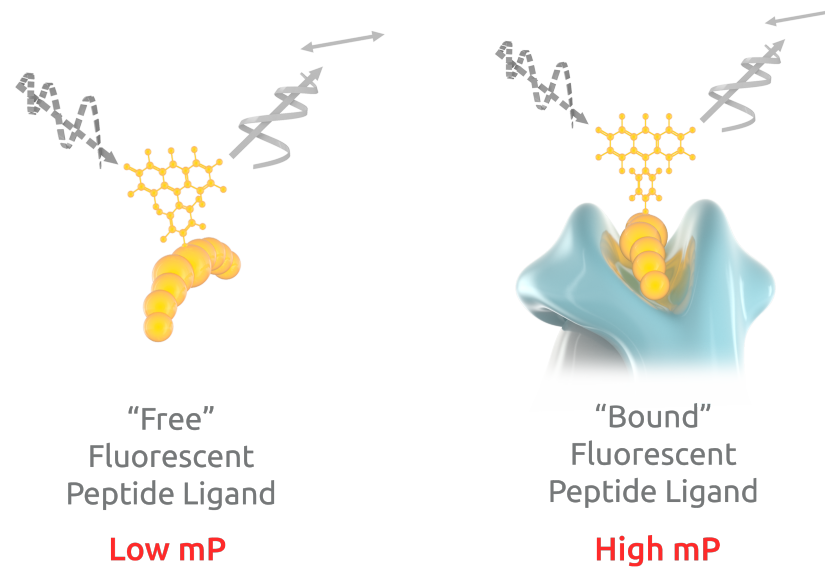
Report PS-B*07:02
May 03, 2020

PI – Rico Buchli, PhD
Tech – Jacob Collard, BS



Competitive Assay Technology

To elaborate the fast and precise molecular binding of a peptide to an HLA molecule, a reference fluorescent-labeled peptide is incubated with activated sHLA in the presence of a peptide competitor and peptide/HLA interaction is monitored over time. Only a simple three step process is required to perform the assay. The activation step (1) is forcing the folded structure into a temporary state of instability, making it amenable for the competitor peptide to be inserted into the groove. The mixing step (2) is providing a fluorescent-labeled tracer peptide (yellow) and test peptide (blue) for competition. And lastly, the measuring step (3) where data is collected, and a positive response will occur when the peptide of interest outcompetes the labeled peptide tracer. A negative response will take place when the peptide of interest has no binding characteristics and only the tracer is assembling with the sHLA.



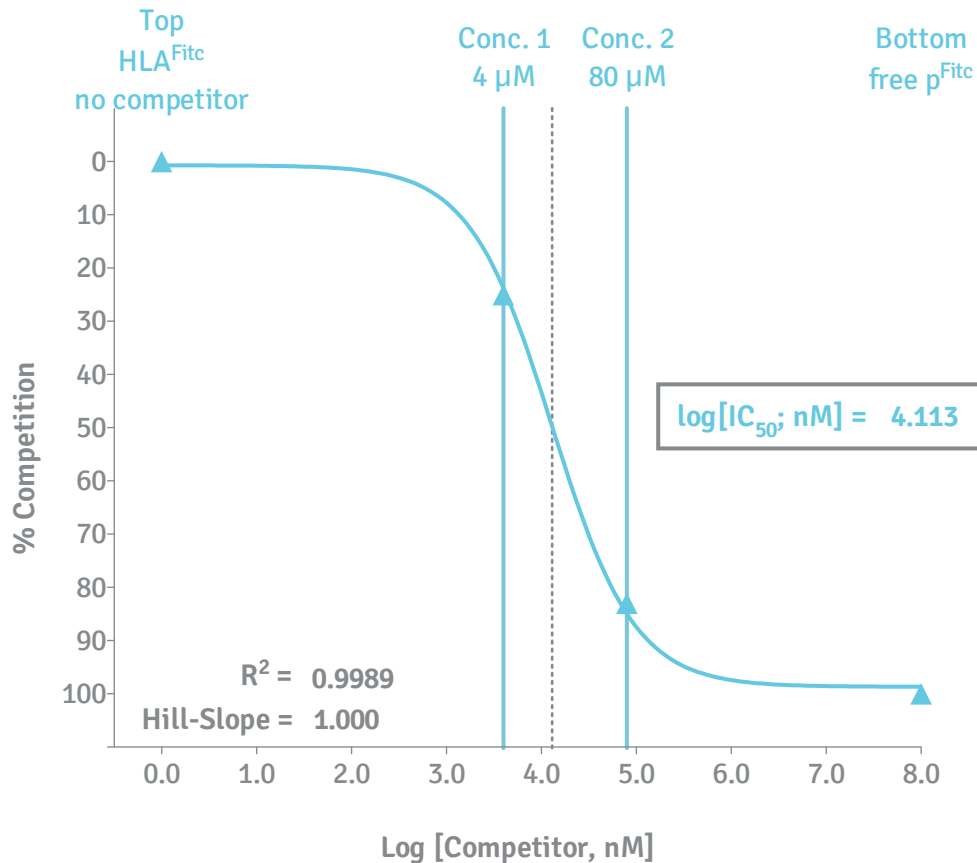
Fluorescence Polarization Technology

Fluorescence polarization (FP) is unique among methods used to analyze molecular binding events because it allows the instantaneous measurement of the ratio between free and bound labeled ligand in solution without any separation steps. The technology is based on the principle that if a fluorescent-labeled peptide binds to the sHLA molecule of higher molecular weight, polarization values will increase due to the slower molecular rotation of the bound probe.

Screening Principle [4-point]

Peptide Epitope Screening Competition Assay

4 pt IC_{50}



A 4-point screen generates a reduced dose-response curve that allows the approximation of $\log IC_{50}$ values based on a limited set of four experimental data points.

Two of the experimental values describe either the bottom of the dose-response curve defined by the free fluorescence peptide tracer [pFitc] or the top value obtained by Fitc-bound HLA with no competitor.

The remaining two screening concentrations are properly spaced to capture the range of affinity differences to generate approximations of $\log IC_{50}$ values.

Within this study, approximations of inhibitory concentrations are determined by incubating sHLA with a labeled reference peptide in the presence of two concentrations of your competitor peptide. A calculated $\log IC_{50}$ value is reported to you as measure of the effectiveness of the competing test peptide. Affinity categories shown will prioritize your $\log IC_{50}$ values into high, medium, or low affinity binders. This approach is mainly used to eliminate none binders which need not be further investigated.

Dose dependent inhibition is a logarithmic phenomenon with a sigmoid function, and as such the applicable format to view and report the data is in $\log IC_{50}$ format.

The delivered results will allow you to judge the T cell peptide epitope candidate's immunogenic potential and value in the development of novel immunotherapies. Relative affinities of multiple peptide ligands for the same HLA receptor can be easily compared. Peptides with higher affinity are more likely to be suitable T cell epitopes and are preferred over the peptides with lower affinity.

Our approach ranks the identified peptides to prioritize epitopes with greatest potential based on preset affinity categories. Our classification of the peptide-binding affinity into high (H), medium (M), low (L), and very low (VL) is comparable to the classifications set by other investigators that have used the same reference peptides [[Buchli et al. 2005](#)].

Affinity Categories

High Affinity	Medium Affinity	Low Affinity	Very Low Affinity	No Binder	
<	3.700	4.700	5.500	6.000	log(IC ₅₀ ; nM)
3.700	4.700	5.500	6.000	>	
<	5,000	50,000	350,000	1,000,000	IC ₅₀ (nM)
5,000	50,000	350,000	1,000,000	>	

Data Overview Report



MHC Peptide Sequence	Internal Name	HLA Allele	log[IC ₅₀]	Binding Category	R ²	Hill Slope	Peptide Length	Exp #	Execution Date
			log[(nM)]				(aa)		
IPSYKKLIM	C1(B*07:02)	B*07:02	2.337	High Affinity	0.9985	-0.774	9	PS200	10/10/20
SLFRAVITK	C2(A*03:01)	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
AAAAAAAAA	Peptide #1	B*07:02	2.685	High Affinity	0.9995	-0.626	9	PS200	10/10/20
BBBBBBBBB	Peptide #2	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
CCCCCCCCC	Peptide #3	B*07:02	5.492	Low Affinity	0.9998	-0.618	9	PS200	10/10/20
DDDDDDDDD	Peptide #4	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
EEEEEEEEE	Peptide #5	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
FFFFFFFFF	Peptide #6	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
GGGGGGGGG	Peptide #7	B*07:02	2.412	High Affinity	0.9993	-0.785	9	PS200	10/10/20
HHHHHHHHH	Peptide #8	B*07:02	4.340	Medium Affinity	1	1.000	10	PS200	10/10/20
IIIIIIIII	Peptide #9	B*07:02	2.661	High Affinity	0.9987	-0.567	9	PS200	10/10/20
JJJJJJJJJ	Peptide #10	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
KKKKKKKKK	Peptide #11	B*07:02	2.387	High Affinity	0.9953	-0.613	9	PS200	10/10/20
LLLLLLLLL	Peptide #12	B*07:02	5.922	Very Low Affinity	0.99	-0.364	10	PS200	10/10/20
MMMMMMMMM	Peptide #13	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
NNNNNNNNN	Peptide #14	B*07:02	5.467	Low Affinity	1	1.000	10	PS200	10/10/20
OOOOOOOOO	Peptide #15	B*07:02	4.557	Medium Affinity	1	-0.549	9	PS200	10/10/20
PPPPPPPPP	Peptide #16	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
QQQQQQQQQ	Peptide #17	B*07:02	3.432	High Affinity	0.9999	-0.579	9	PS200	10/10/20
RRRRRRRRR	Peptide #18	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
SSSSSSSSS	Peptide #19	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
TTTTTTTTT	Peptide #20	B*07:02	4.645	Medium Affinity	0.9934	1.000	10	PS200	10/10/20
UUUUUUUUU	Peptide #21	B*07:02	4.137	Medium Affinity	0.999	-0.348	9	PS200	10/10/20
VVVVVVVVV	Peptide #22	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
WWWWWWWWW	Peptide #23	B*07:02	3.519	High Affinity	0.9998	-0.477	9	PS200	10/10/20
XXXXXXXXXX	Peptide #24	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
YYYYYYYYY	Peptide #25	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
ZZZZZZZZZ	Peptide #26	B*07:02	3.274	High Affinity	0.9997	-0.490	10	PS200	10/10/20
ABABABABA	Peptide #27	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
CDCDCDCDC	Peptide #28	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
EFEFEFEFE	Peptide #29	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
GHGHHGHGH	Peptide #30	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20

Peptide Epitope Binding Results

MHC Peptide Sequence	Internal Name	HLA Allele	log[IC ₅₀]	Binding Category	R ²	Hill Slope	Peptide Length	Exp #	Execution Date
			log[(nM)]				(aa)		
IPSYKKLIM	C1(B*07:02)	B*07:02	2.337	High Affinity	0.9985	-0.774	9	PS200	10/10/20
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AAAAAAAAA	Peptide #1	B*07:02	2.685	High Affinity	0.9995	-0.626	9	PS200	10/10/20
ZZZZZZZZZ	Peptide #26	B*07:02	3.274	High Affinity	0.9997	-0.490	10	PS200	10/10/20
QQQQQQQQQ	Peptide #17	B*07:02	3.432	High Affinity	0.9999	-0.579	9	PS200	10/10/20
WWWWWWWWW	Peptide #23	B*07:02	3.519	High Affinity	0.9998	-0.477	9	PS200	10/10/20
UUUUUUUUU	Peptide #21	B*07:02	4.137	Medium Affinity	0.999	-0.348	9	PS200	10/10/20
HHHHHHHHH	Peptide #8	B*07:02	4.340	Medium Affinity	1	1.000	10	PS200	10/10/20
OOOOOOOOO	Peptide #15	B*07:02	4.557	Medium Affinity	1	-0.549	9	PS200	10/10/20
TTTTTTTTT	Peptide #20	B*07:02	4.645	Medium Affinity	0.9934	1.000	10	PS200	10/10/20
NNNNNNNNN	Peptide #14	B*07:02	5.467	Low Affinity	1	1.000	10	PS200	10/10/20
CCCCCCCCC	Peptide #3	B*07:02	5.492	Low Affinity	0.9998	-0.618	9	PS200	10/10/20
LLLLLLLLL	Peptide #12	B*07:02	5.922	Very Low Affinity	0.99	-0.364	10	PS200	10/10/20

FULL DATA SET PROVIDED AS EXCEL WORKSHEET

PS-Results.xlsx

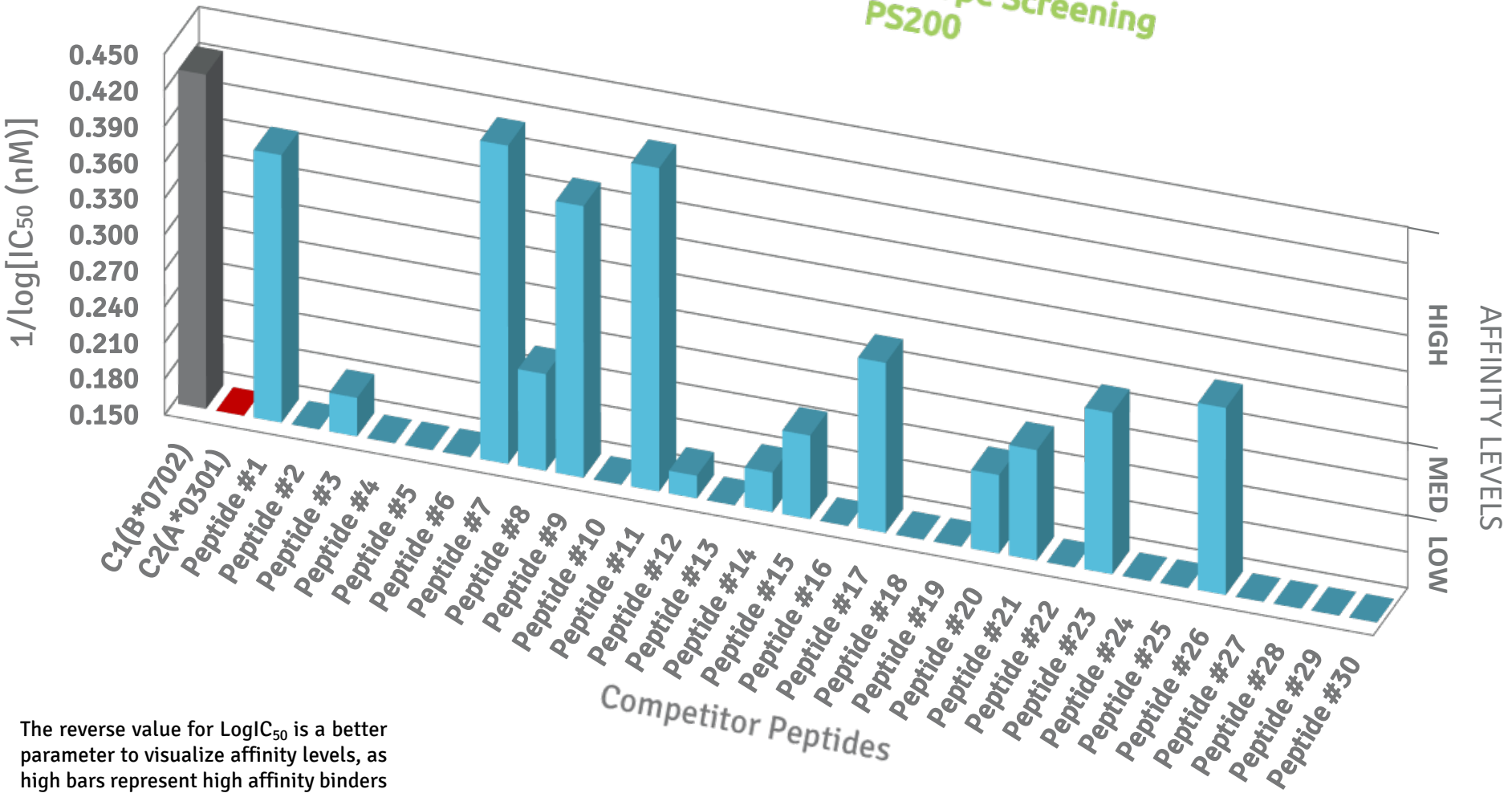
None Binding Results

MHC Peptide Sequence	Internal Name	HLA Allele	log[IC ₅₀]	Binding Category	R ²	Hill Slope	Peptide Length (aa)	Exp #	Execution Date
SLFRAVITK	C2(A*03:01)	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
BBBBBBBBBB	Peptide #2	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
DDDDDDDDDD	Peptide #4	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
EEEEEEEEEE	Peptide #5	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
FFFFFFFFFF	Peptide #6	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
JJJJJJJJJJ	Peptide #10	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
MMMMMMMMMM	Peptide #13	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
PPPPPPPPPP	Peptide #16	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
RRRRRRRRRR	Peptide #18	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
SSSSSSSSSS	Peptide #19	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
VVVVVVVVVV	Peptide #22	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
XXXXXXXXXX	Peptide #24	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
YYYYYYYYYY	Peptide #25	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
ABABABABA	Peptide #27	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
CDCDCDCDCD	Peptide #28	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20
EFEFEFEFE	Peptide #29	B*07:02	None Binder	None Binder	-	-	9	PS200	10/10/20
GHHGHGHGHG	Peptide #30	B*07:02	None Binder	None Binder	-	-	10	PS200	10/10/20

FULL DATA SET PROVIDED AS EXCEL WORKSHEET

PS-Results.xlsx

4Pt Peptide Epitope Screening PS200

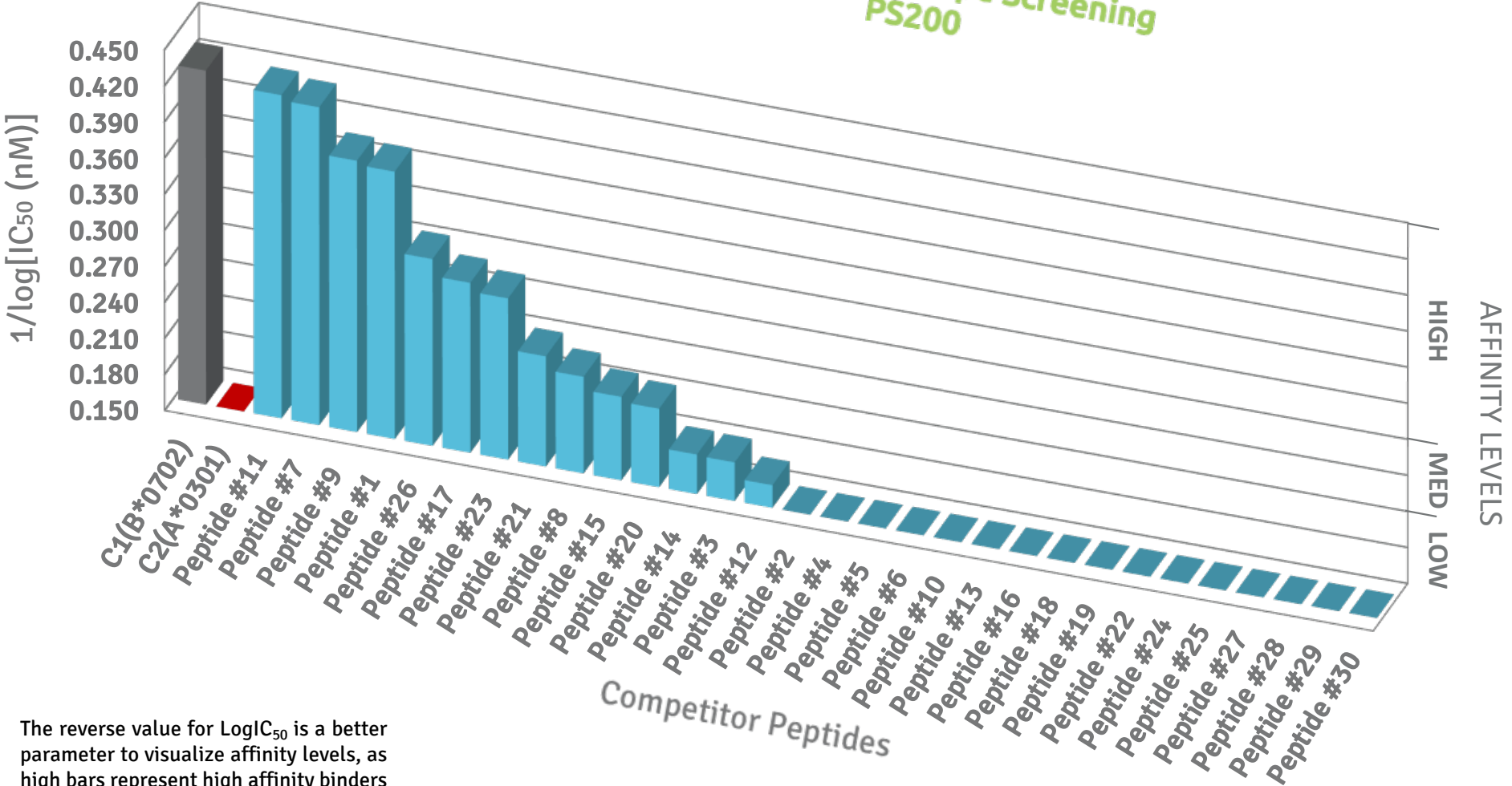


The reverse value for LogIC₅₀ is a better parameter to visualize affinity levels, as high bars represent high affinity binders and vice versa.

Data MAP sorted



4Pt Peptide Epitope Screening
PS200



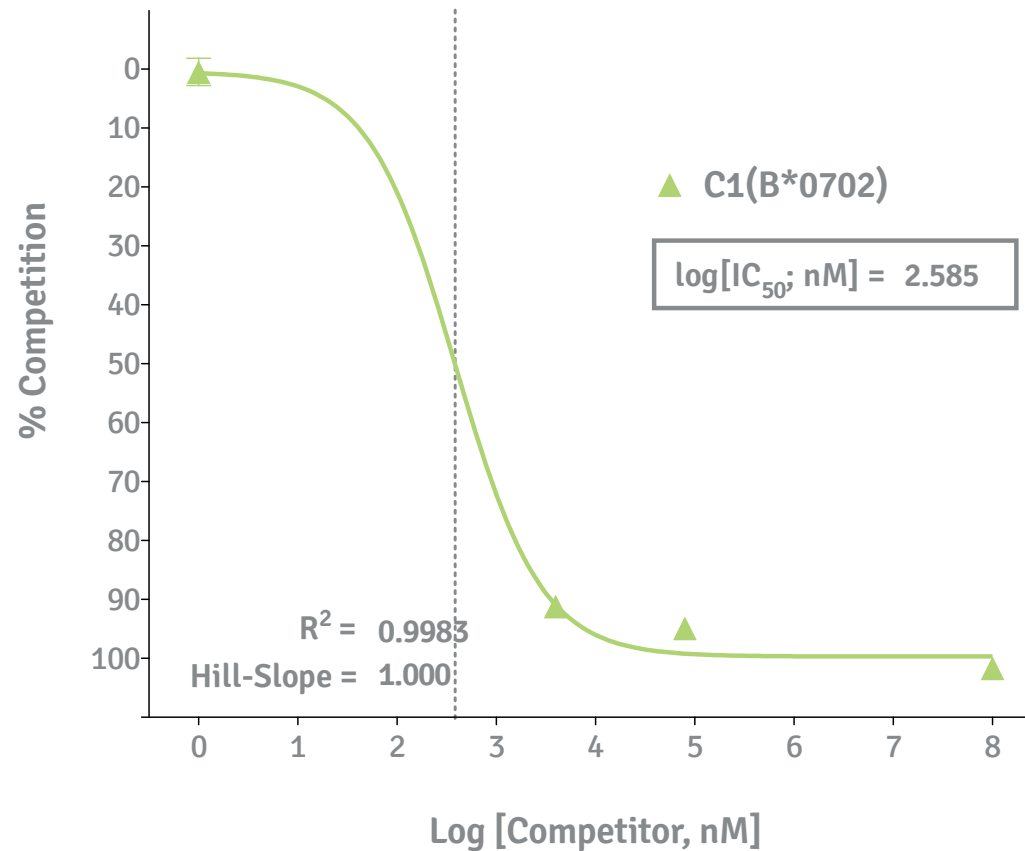
The reverse value for LogIC₅₀ is a better parameter to visualize affinity levels, as high bars represent high affinity binders and vice versa.

IC₅₀ DATA COLLECTION



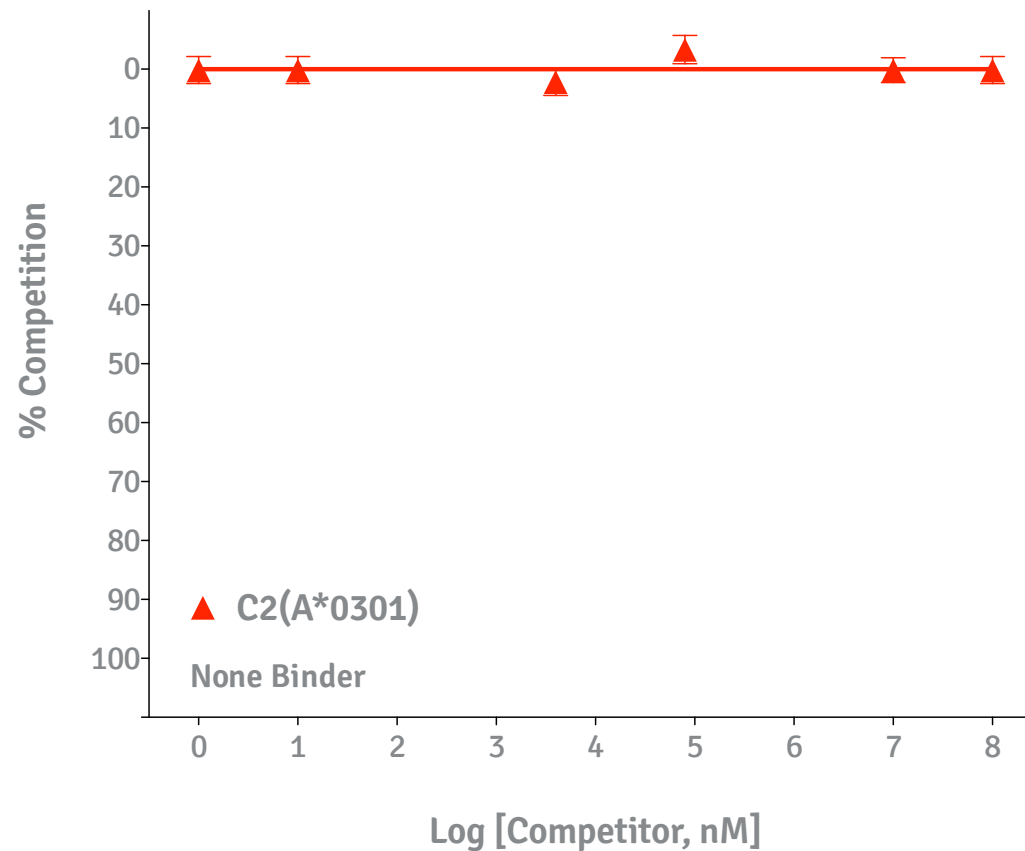
Peptide Epitope Screening Competition Assay PS-B*07:02

IPSYKKLIM
(PS000 +Control)



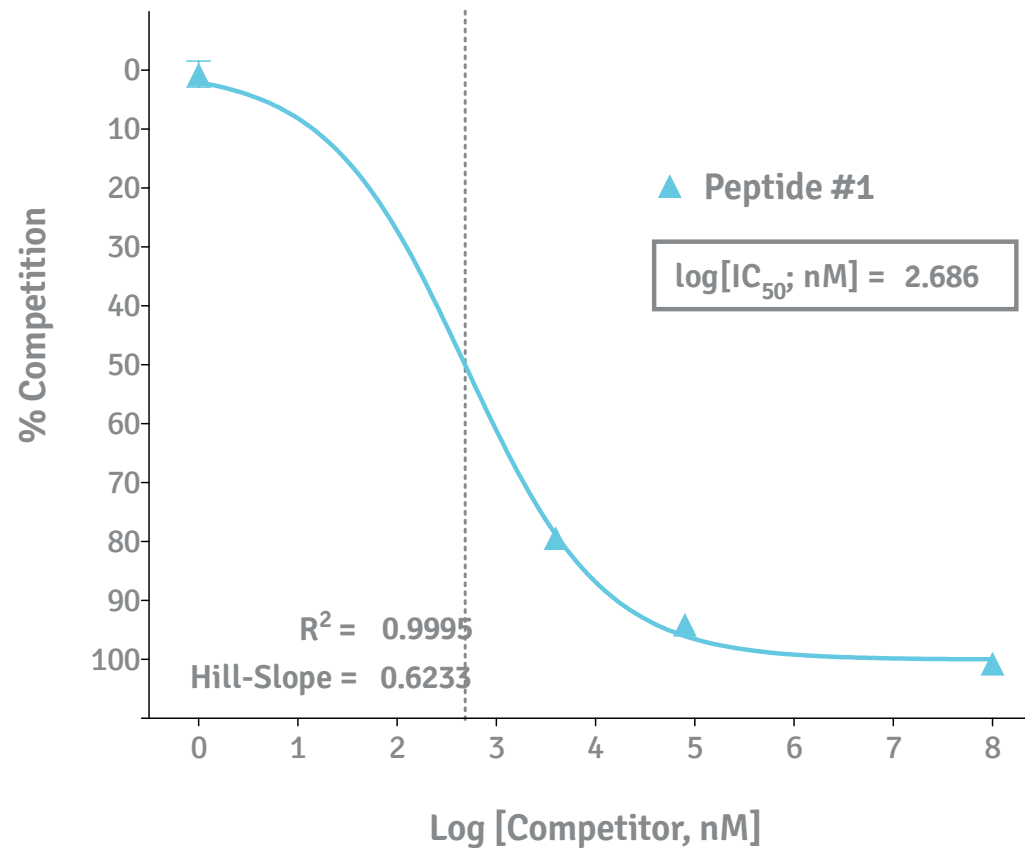
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SLFRAVITK
(PS000 -Control)



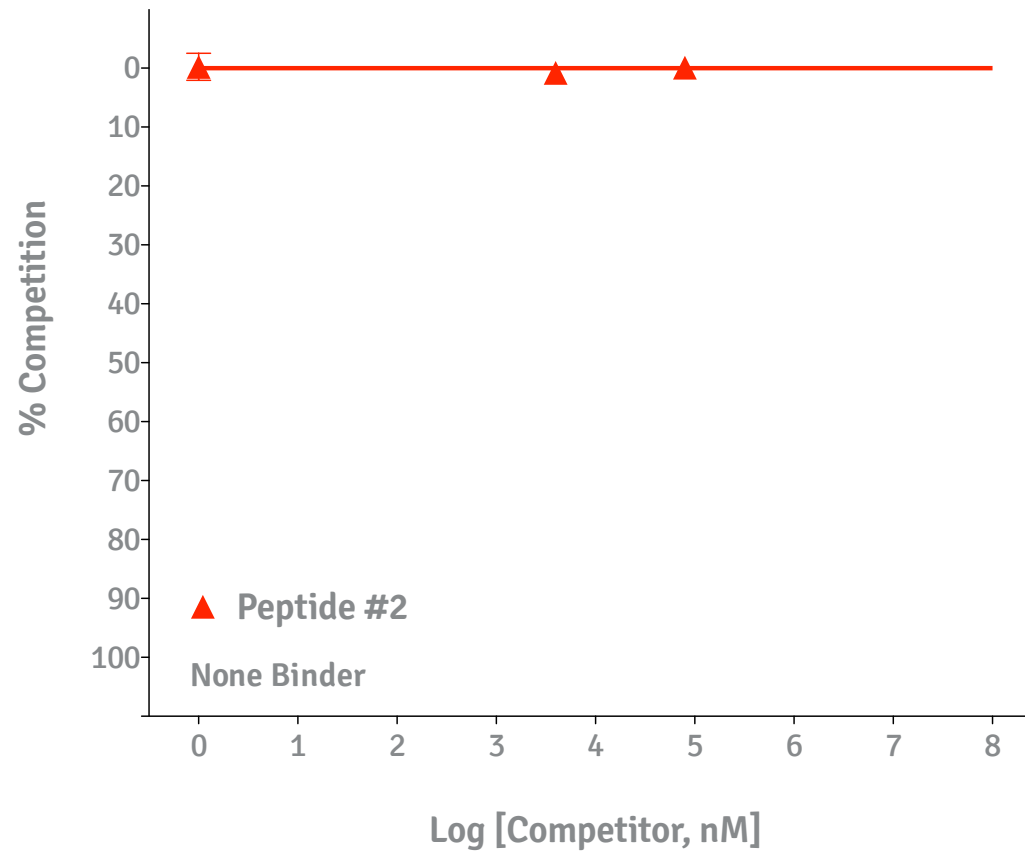
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AAAAAAAAA
(PS000-1)



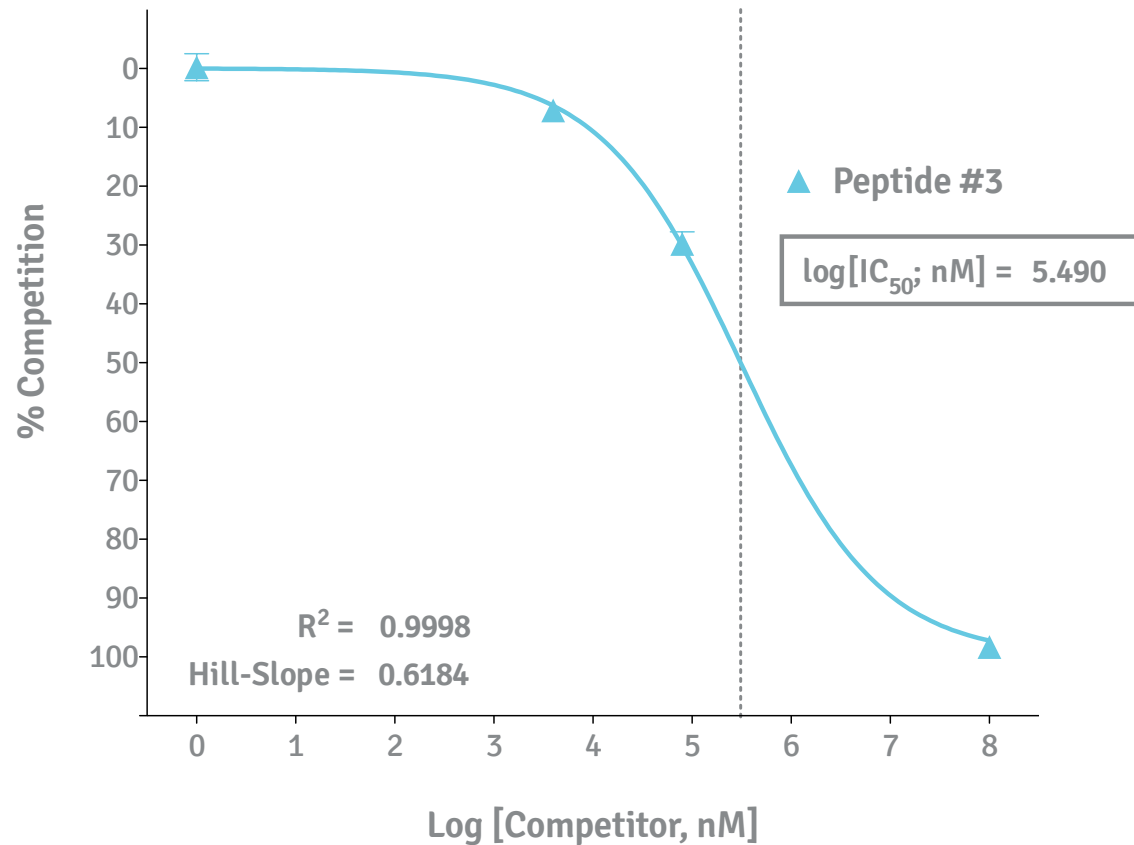
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BBBBBBBBBB
(PS000-2)



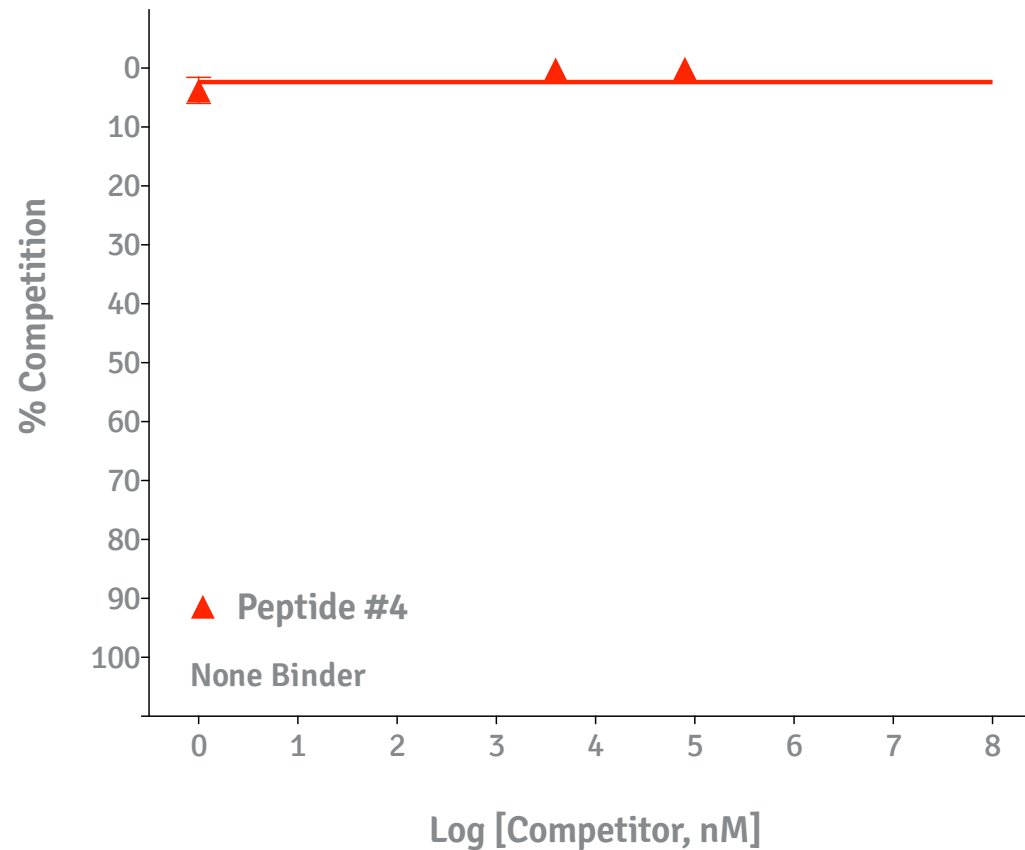
Peptide Epitope Screening Competition Assay PS-B*07:02

CCCCCCCCC
(PS000-3)



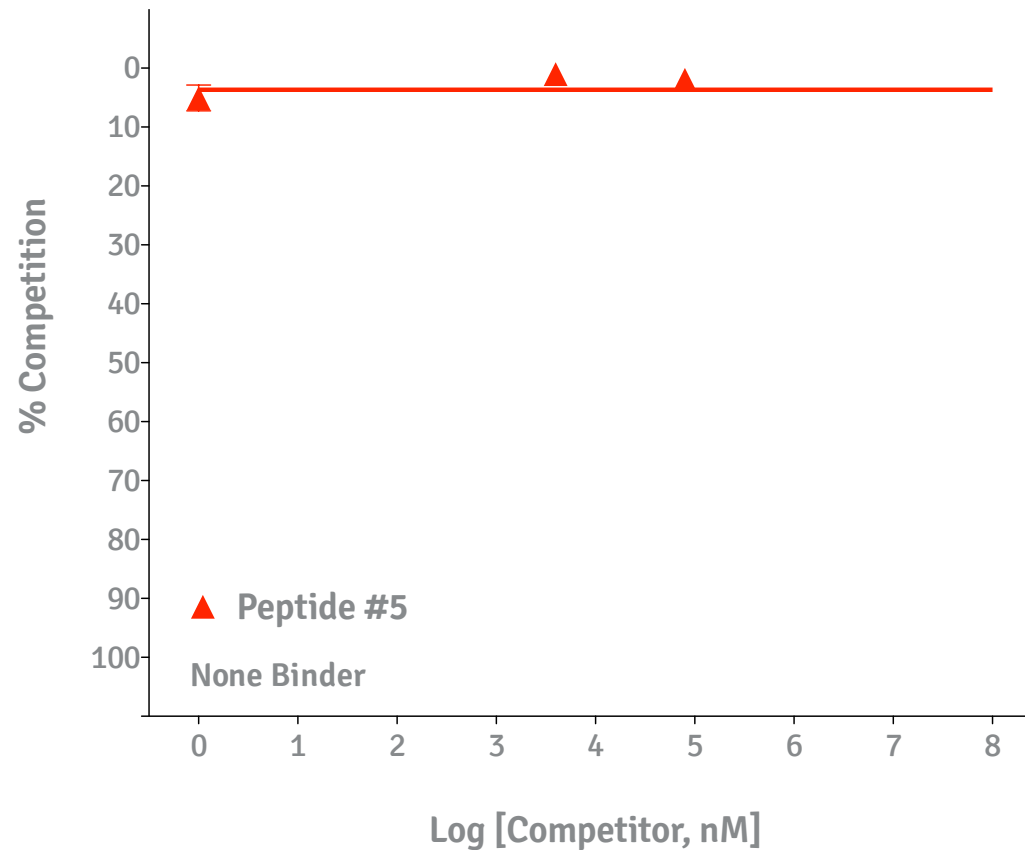
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DDDDDDDDDD
(PS000-4)



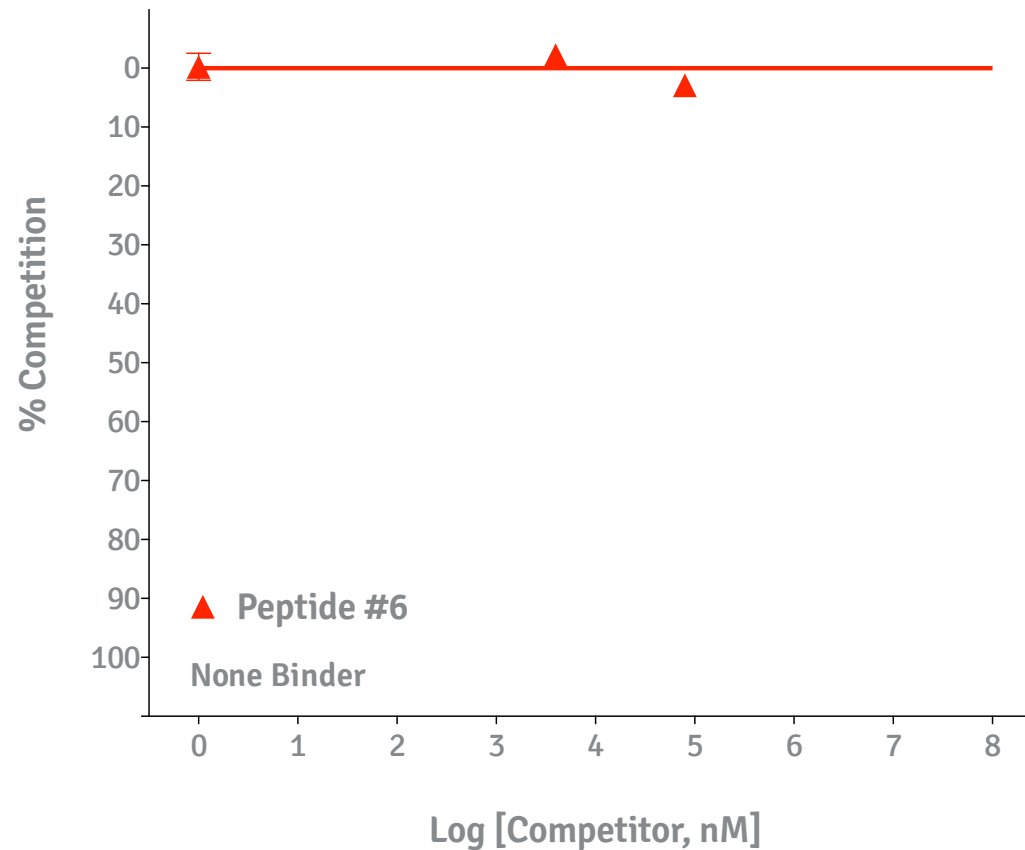
Peptide Epitope Screening Competition Assay PS-B*07:02

EEEEEEEEEE
(PS000-5)



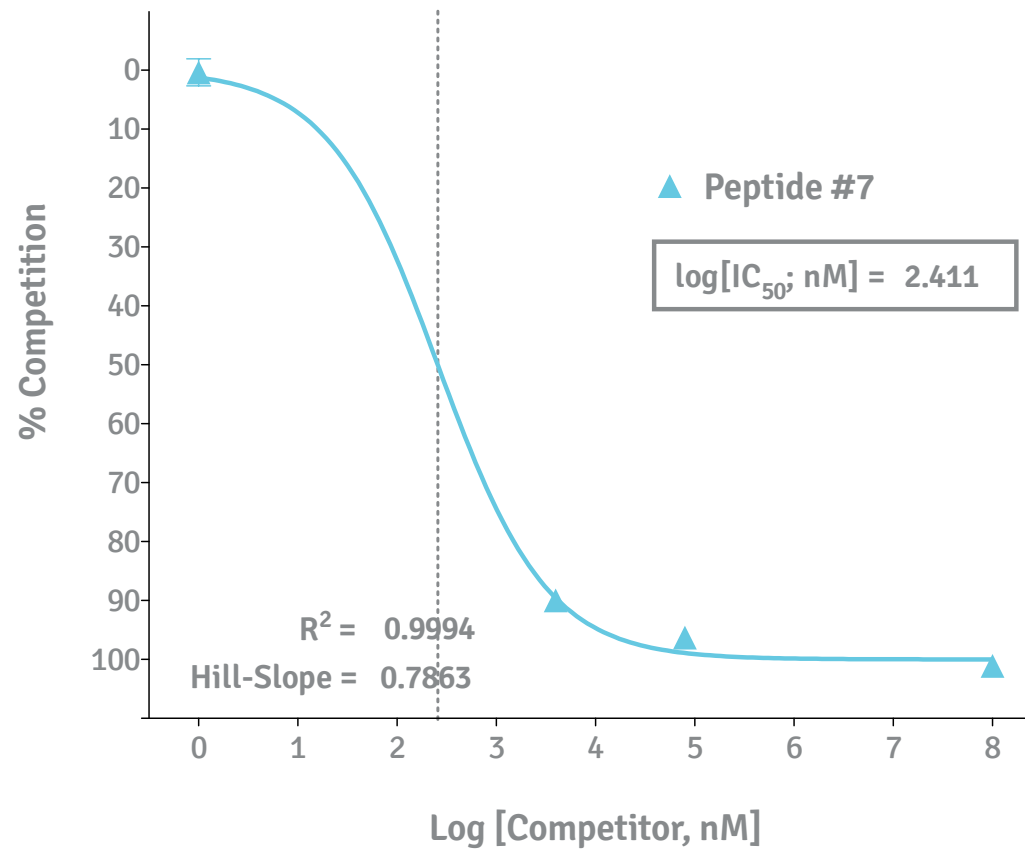
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FFFFFFFFF
(PS000-6)



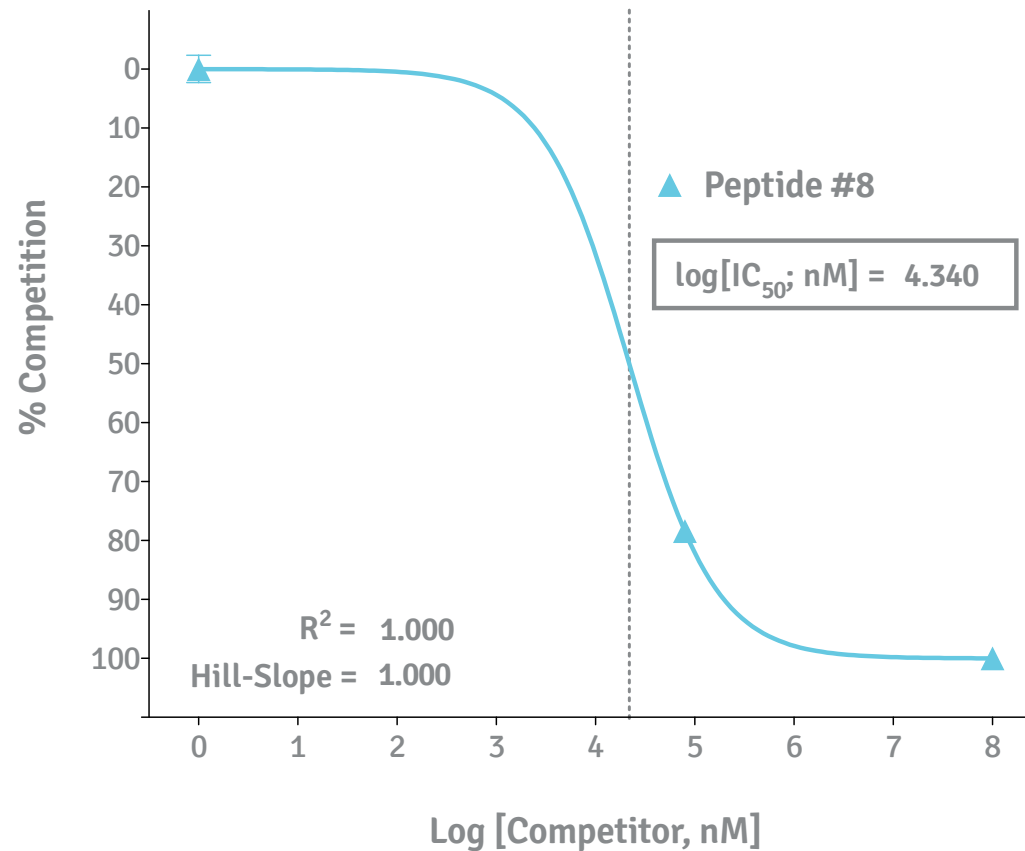
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GGGGGGGGG
(PS000-7)



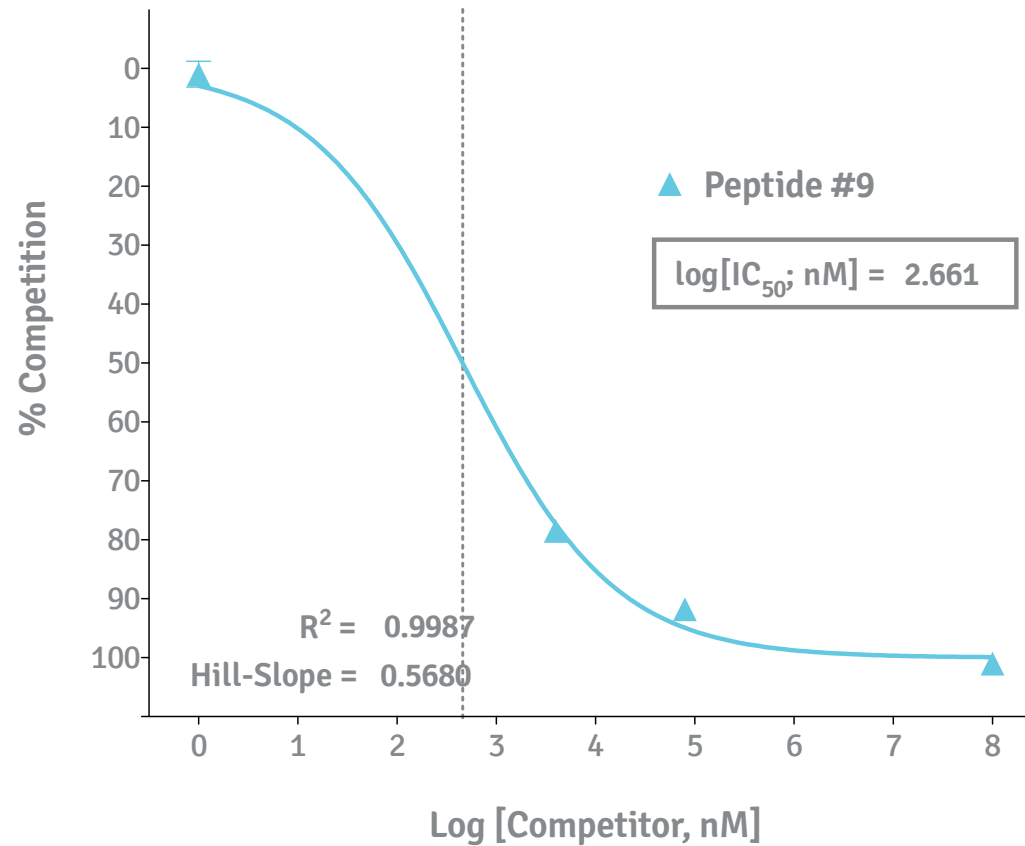
Peptide Epitope Screening Competition Assay PS-B*07:02

HHHHHHHHHH
(PS000-8)



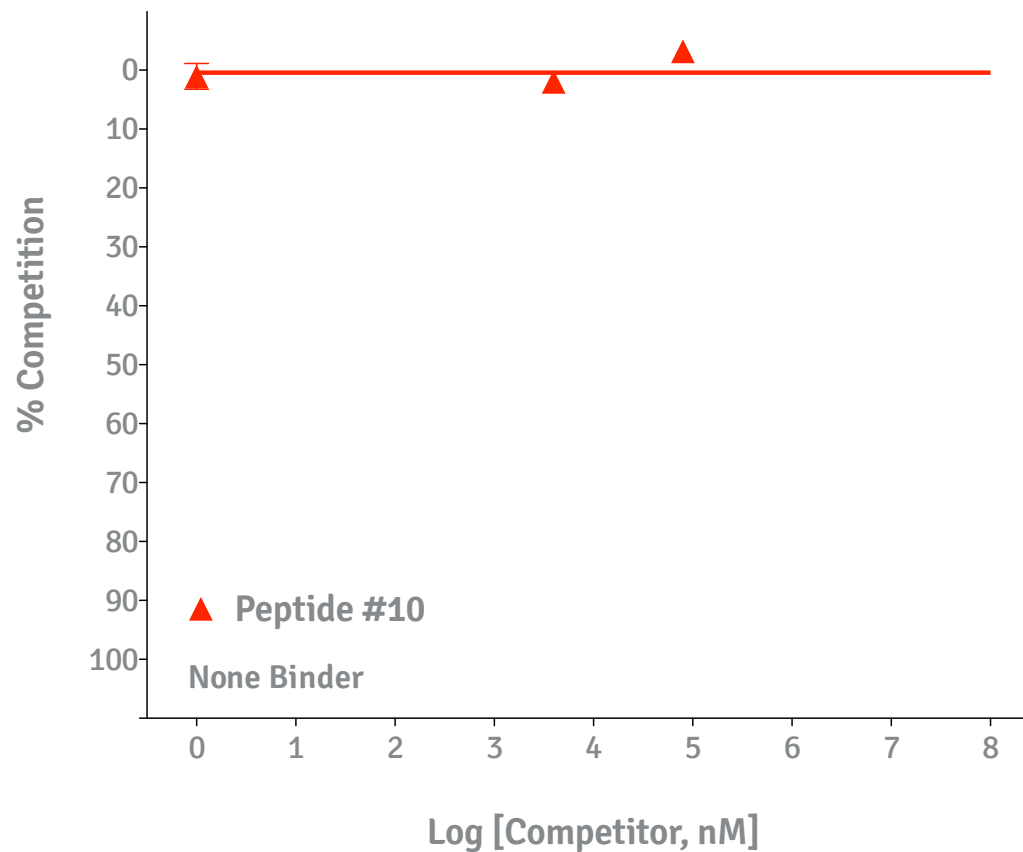
Peptide Epitope Screening Competition Assay PS-B*07:02

|||||||
(PS000-9)



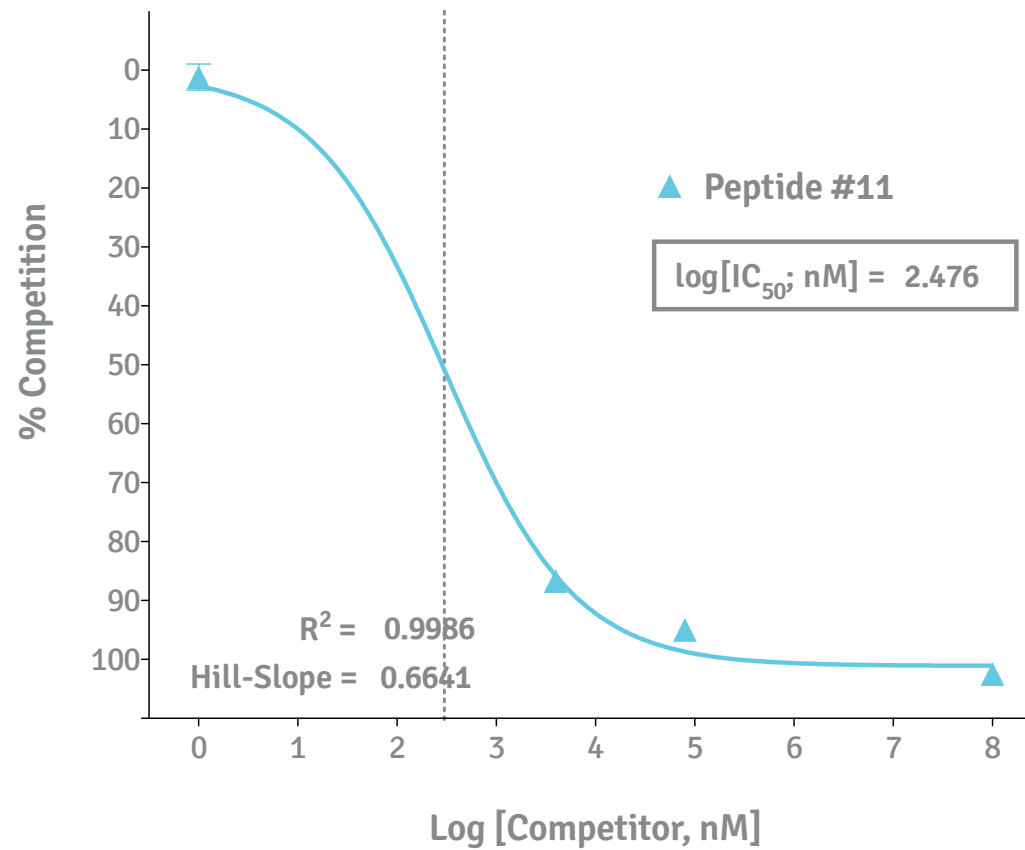
Peptide Epitope Screening Competition Assay PS-B*07:02

JJJJJJJJJJ
(PS000-10)



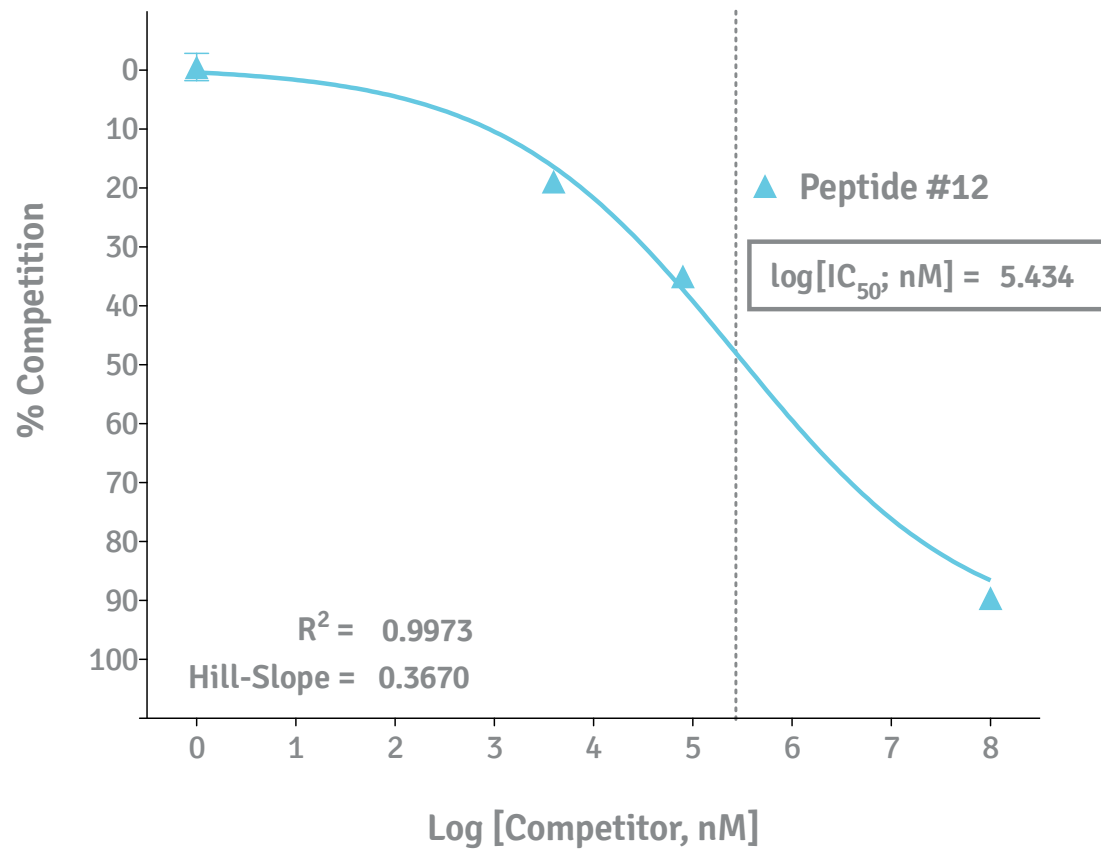
Peptide Epitope Screening Competition Assay PS-B*07:02

KKKKKKKKK
(PS000-11)



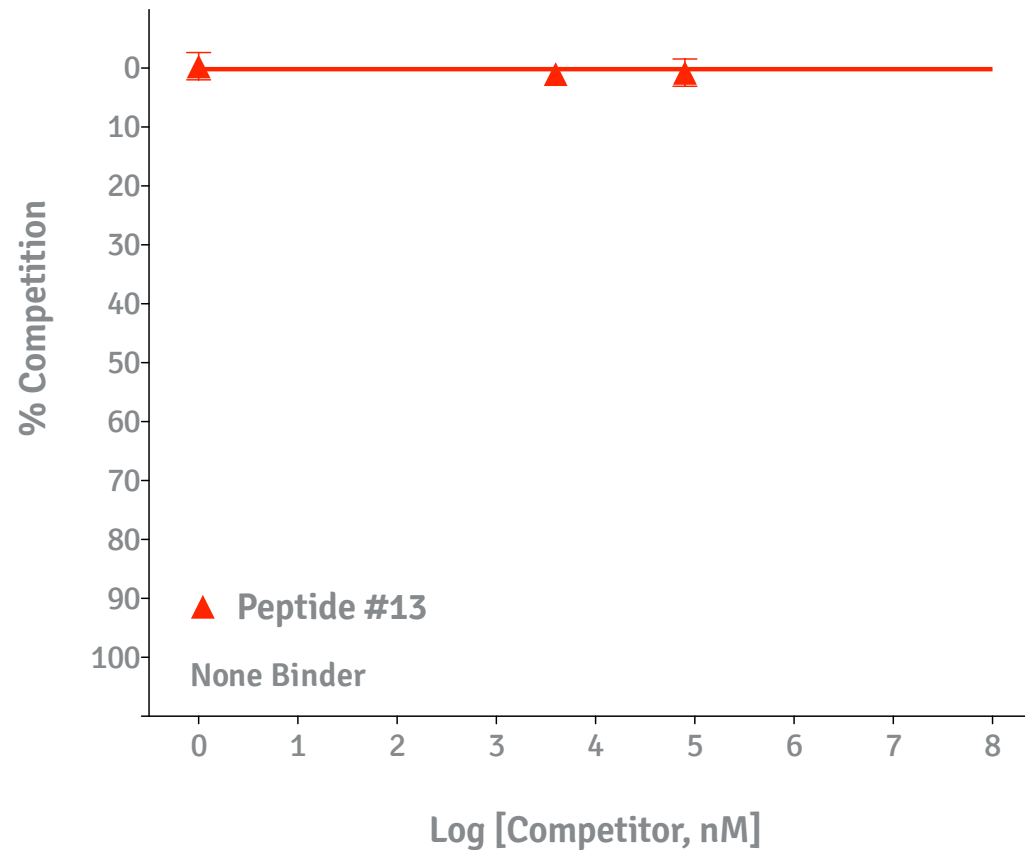
Peptide Epitope Screening Competition Assay PS-B*07:02

LLLLLLLLLL
(PS000-12)



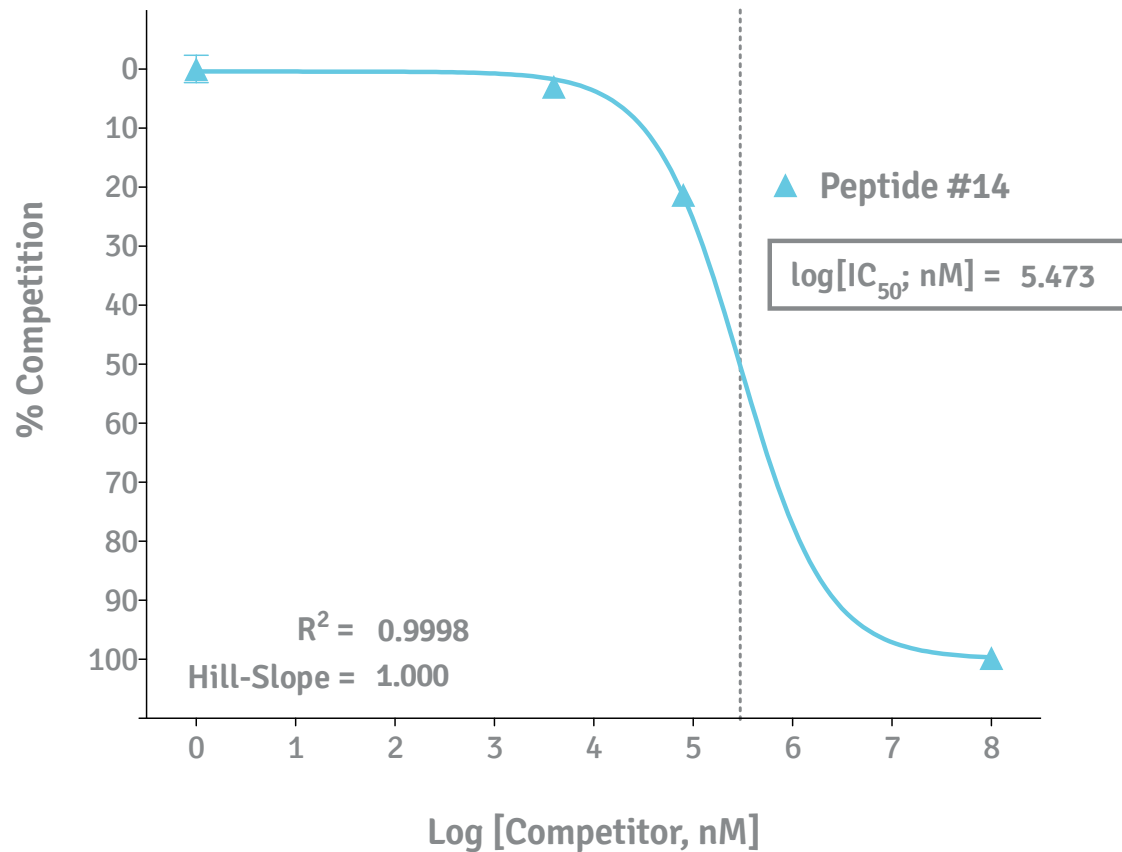
Peptide Epitope Screening Competition Assay PS-B*07:02

MMMMMMMMM
(PS000-13)



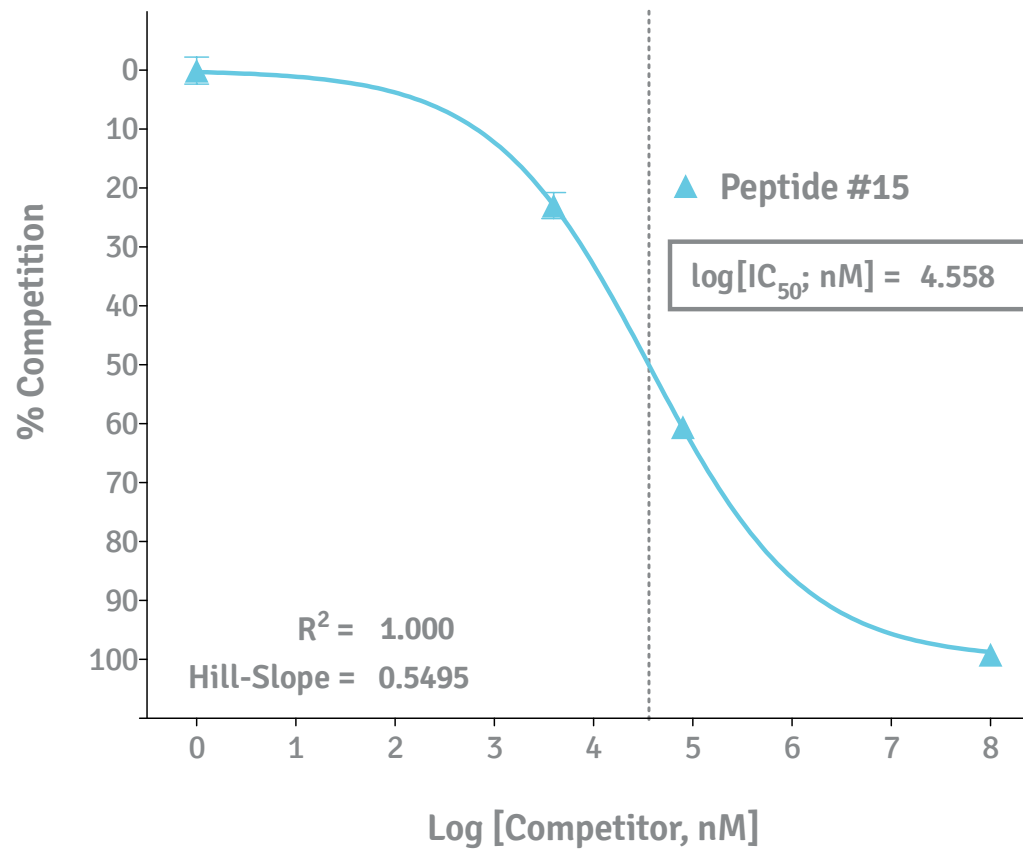
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NNNNNNNNNN
(PS000-14)



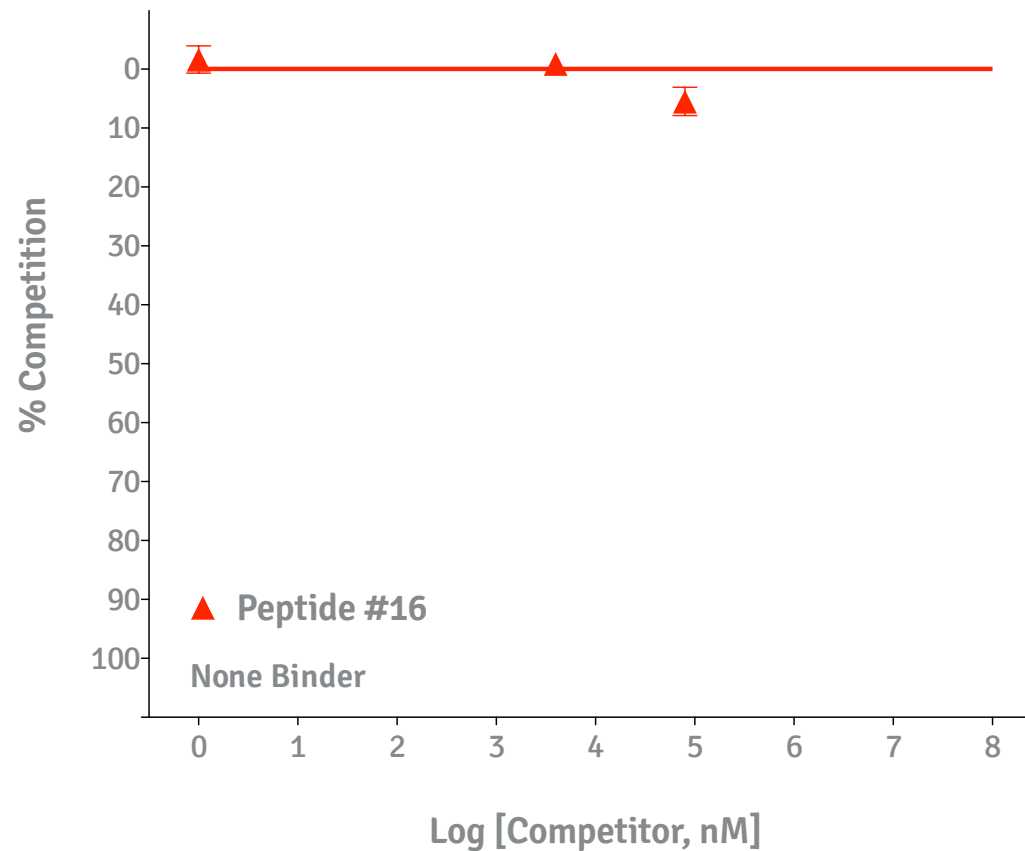
Peptide Epitope Screening Competition Assay PS-B*07:02

OOOOOOOOO
(PS000-15)



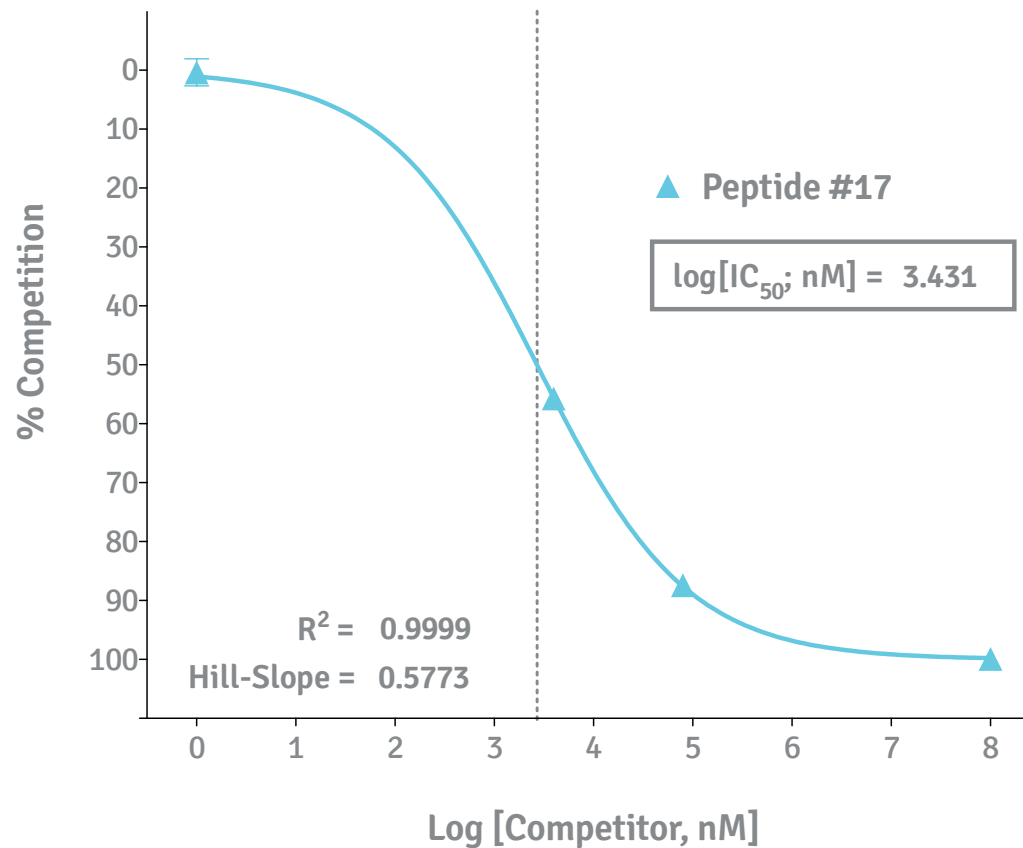
Peptide Epitope Screening Competition Assay PS-B*07:02

PPPPPPPPPP
(PS000-16)



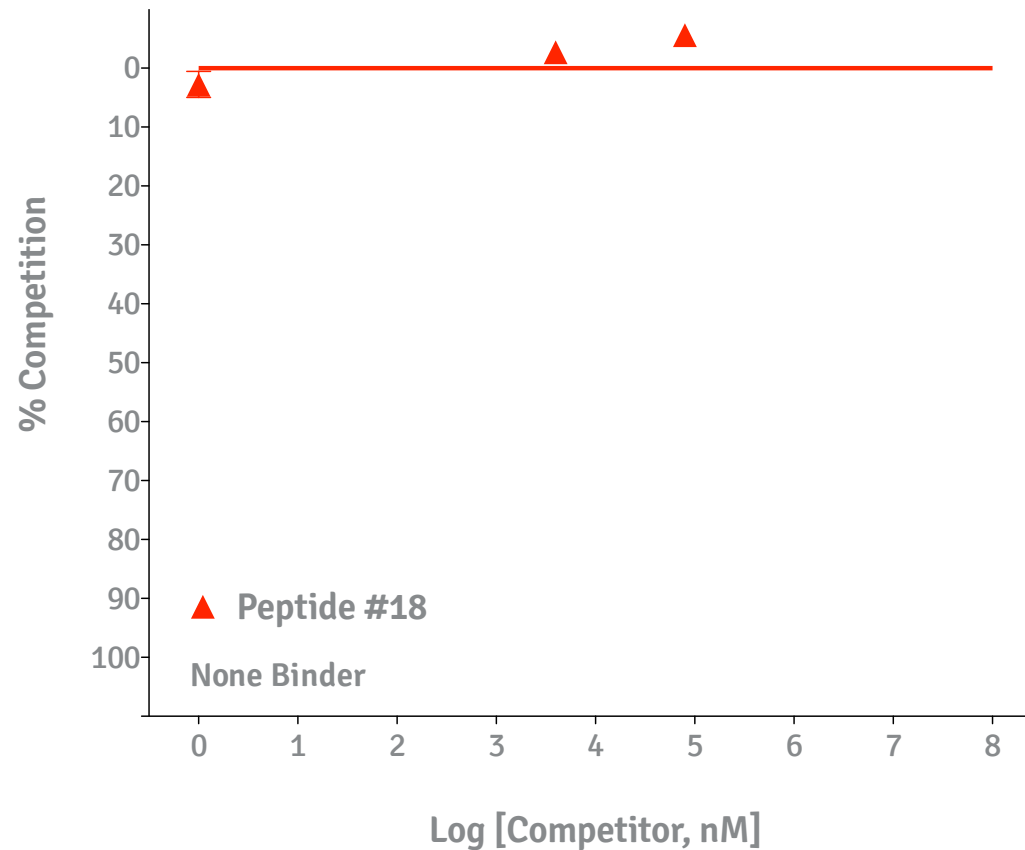
Peptide Epitope Screening Competition Assay PS-B*07:02

QQQQQQQQQQ
(PS000-17)



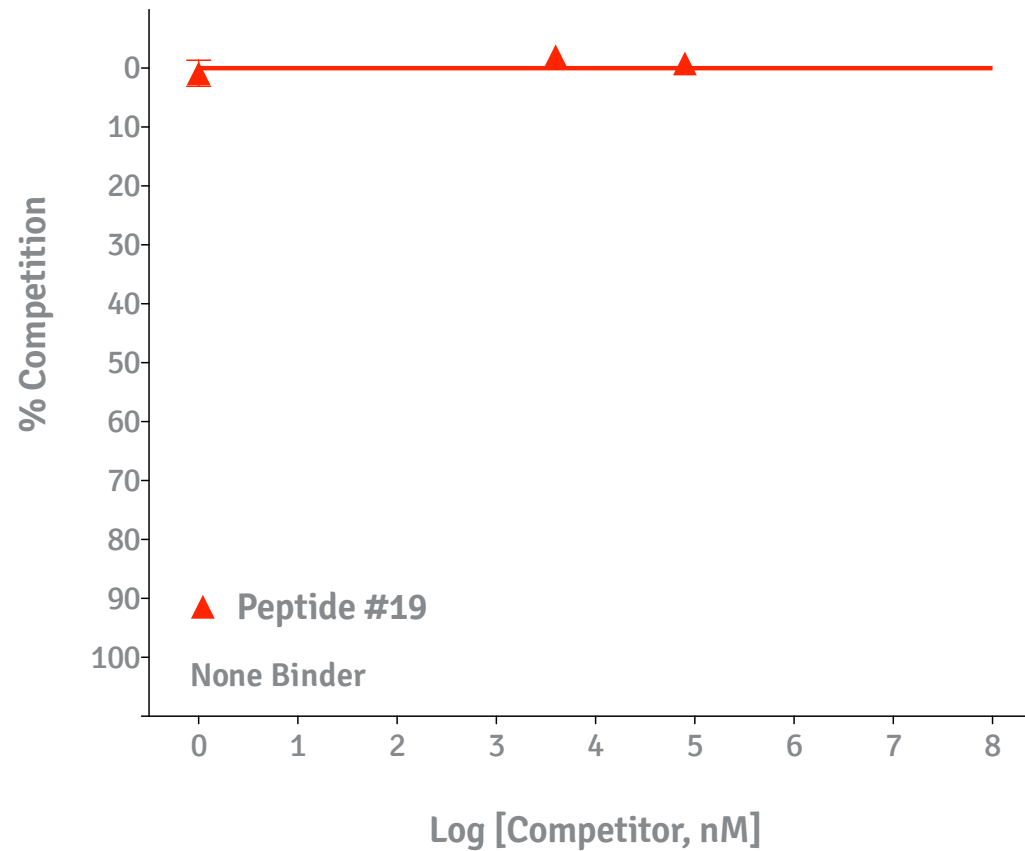
Peptide Epitope Screening Competition Assay PS-B*07:02

RRRRRRRRRR
(PS000-18)



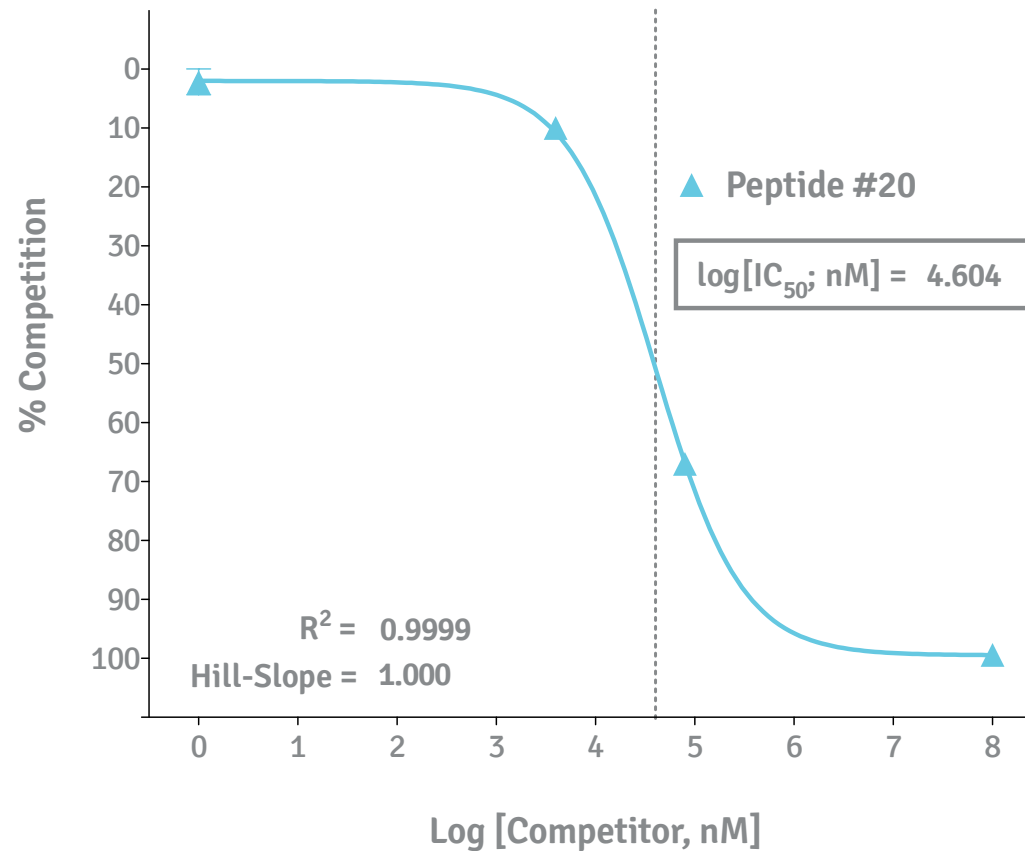
Peptide Epitope Screening Competition Assay PS-B*07:02

SSSSSSSS
(PS000-19)



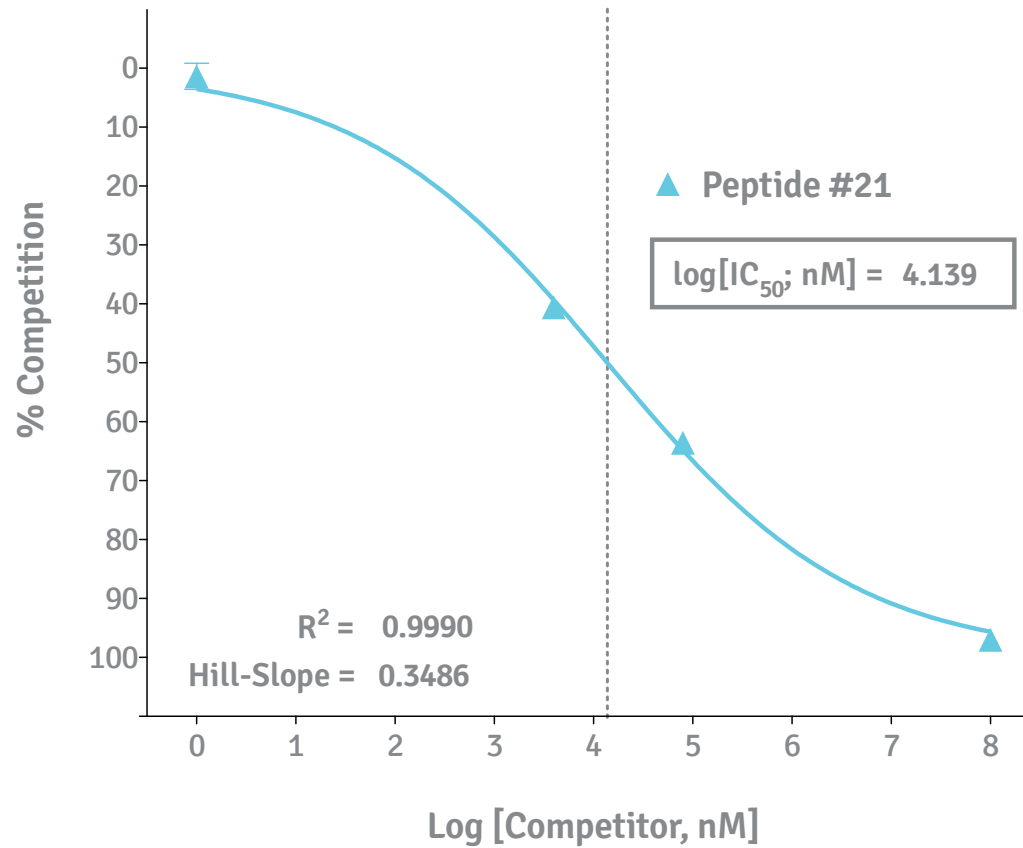
Peptide Epitope Screening Competition Assay PS-B*07:02

TTTTTTTTTT
(PS000-20)



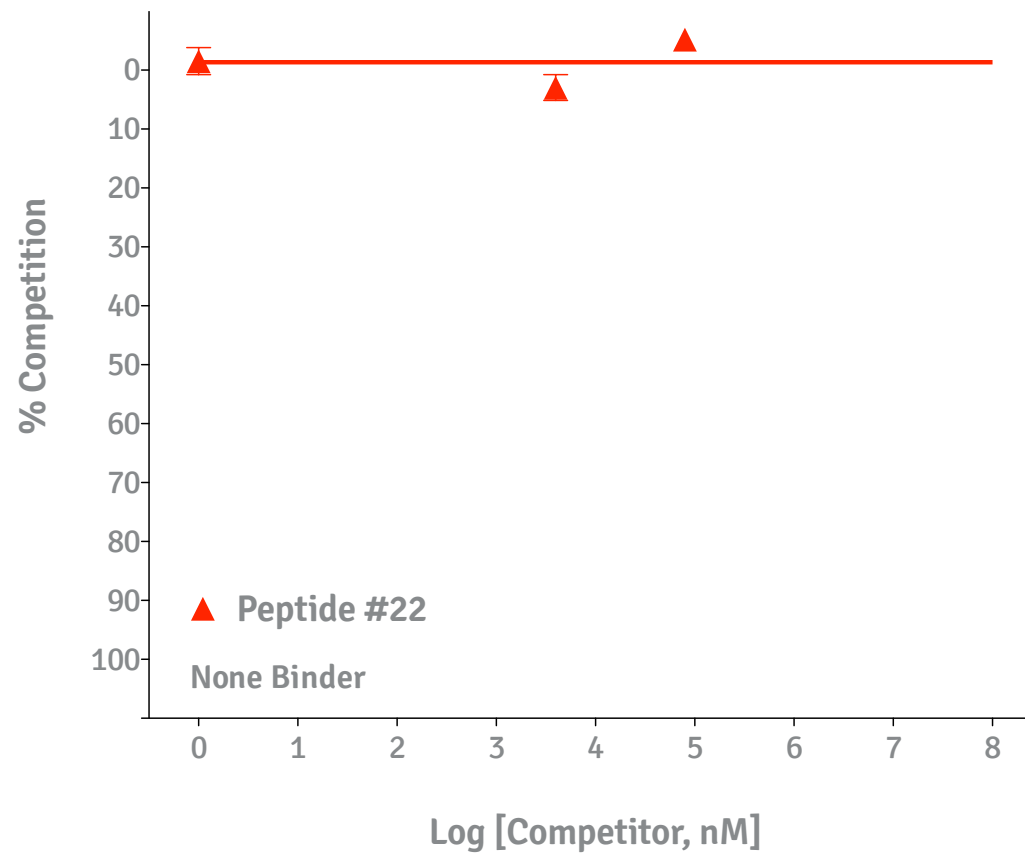
Peptide Epitope Screening Competition Assay PS-B*07:02

UUUUUUUUUU
(PS000-21)



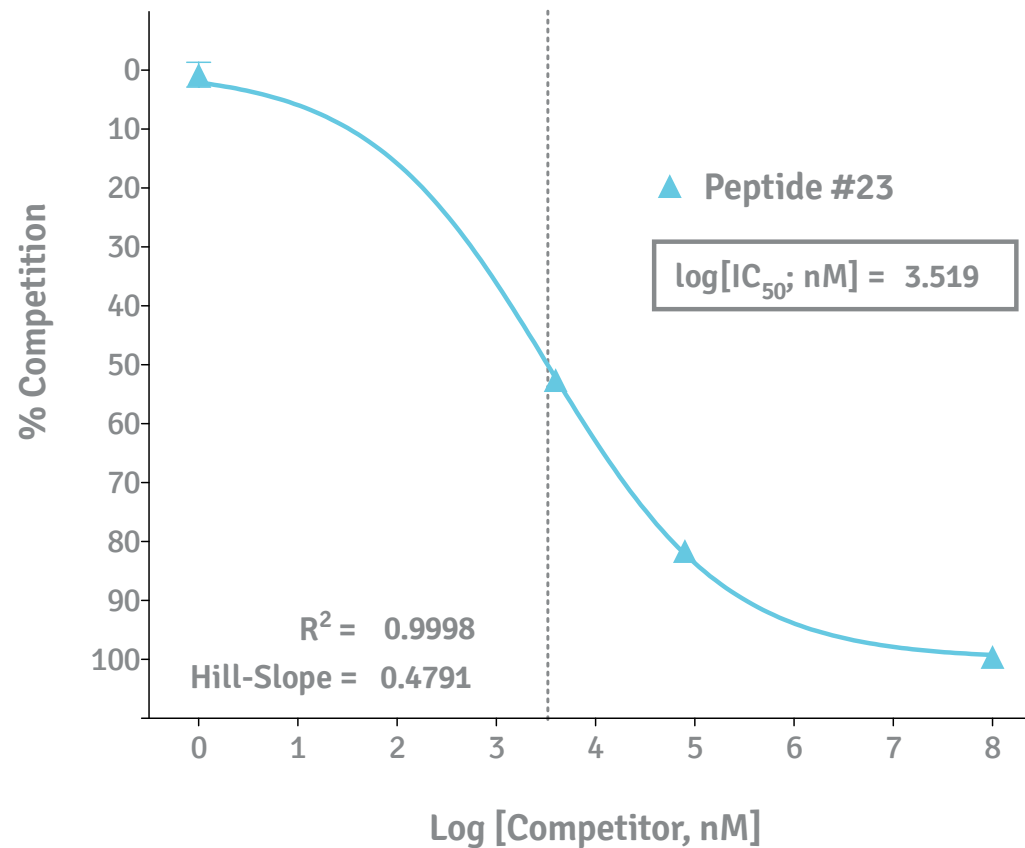
Peptide Epitope Screening Competition Assay PS-B*07:02

VVVVVVVVVVV
(PS000-22)



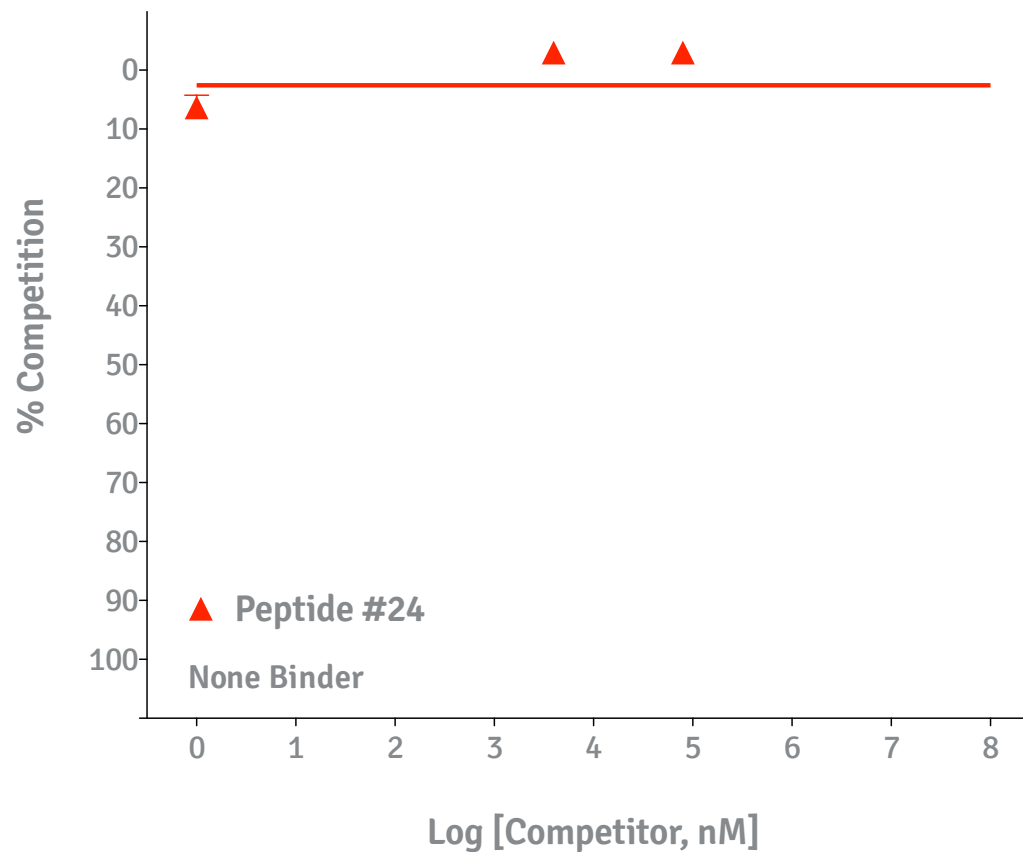
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WWWWWWWWW
(PS000-23)



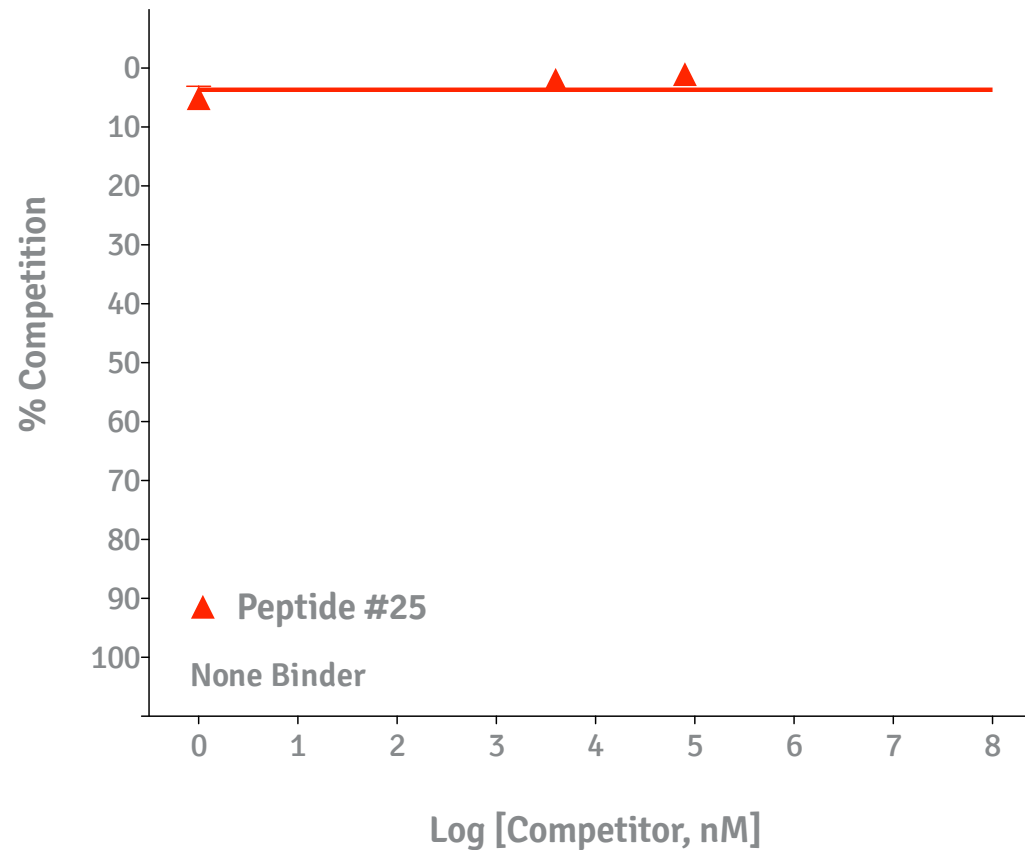
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XXXXXXXXXX
(PS000-24)



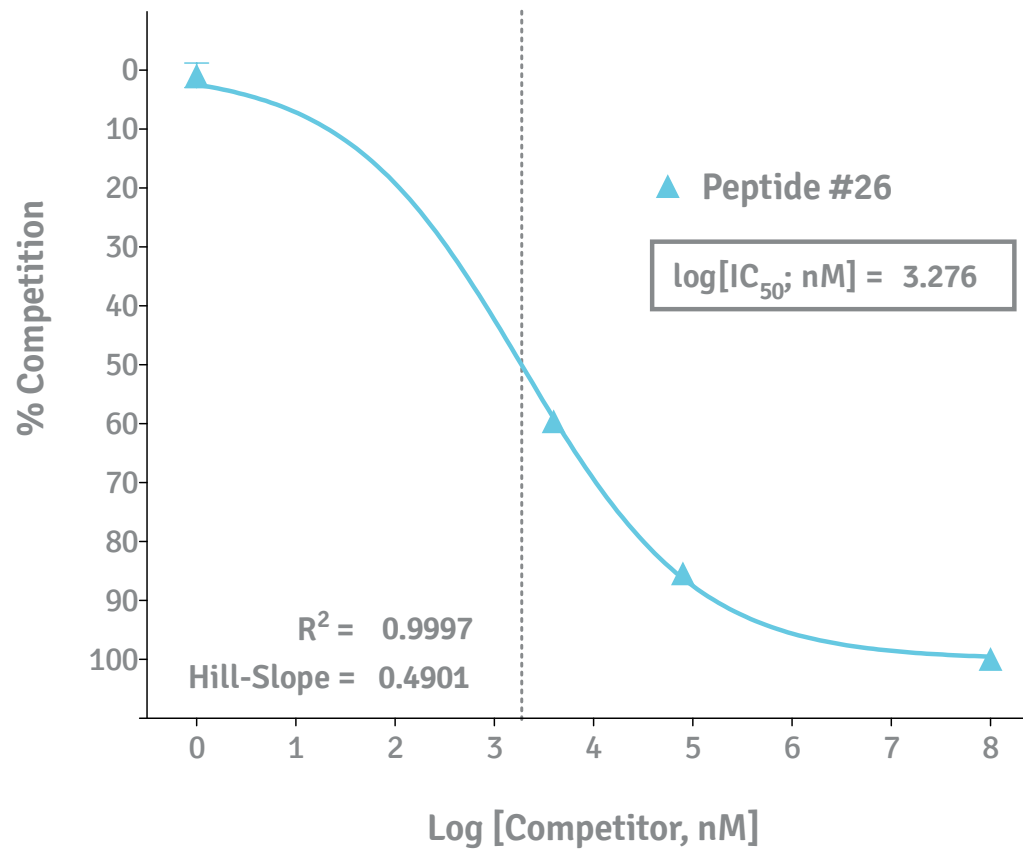
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YYYYYYYYYY
(PS000-25)



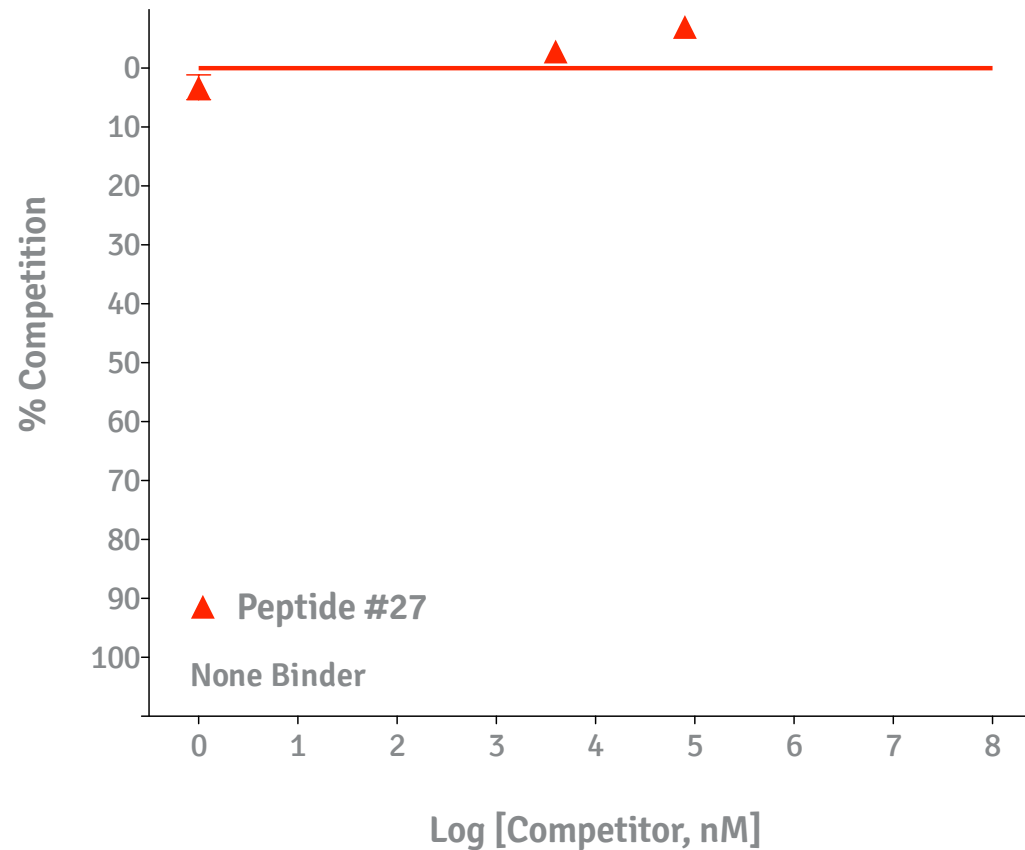
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ZZZZZZZZZZ
(PS000-26)



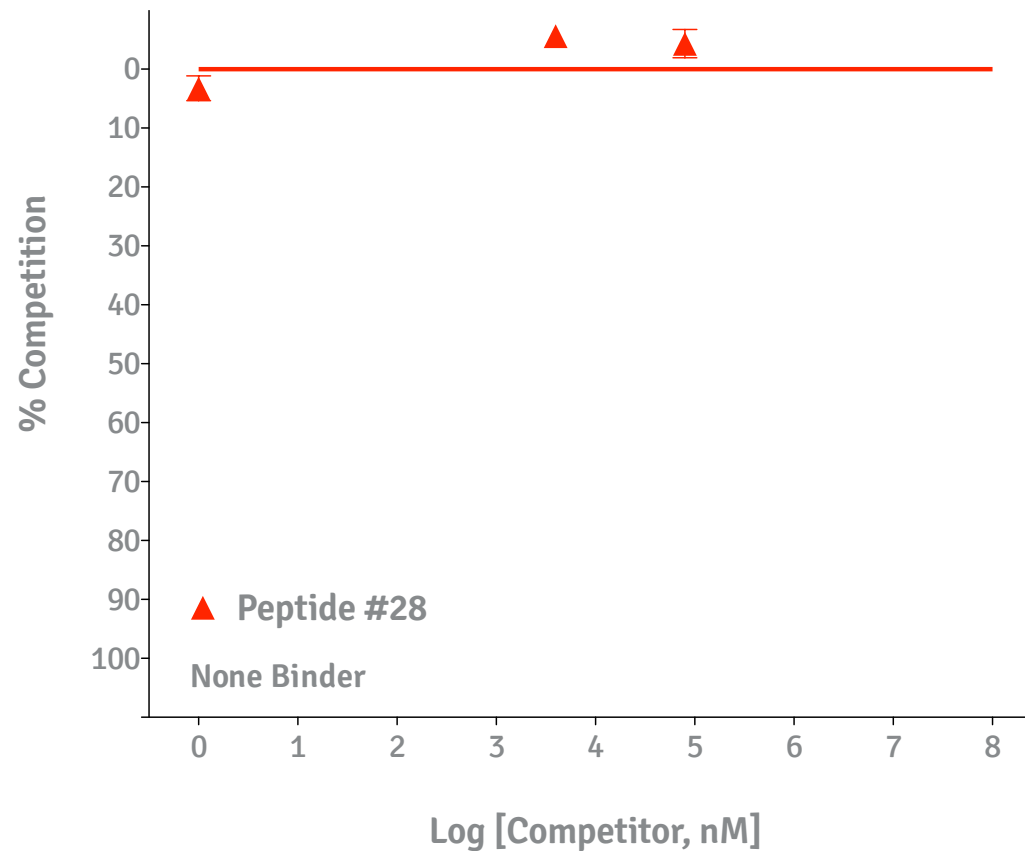
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ABABABABA
(PS000-27)



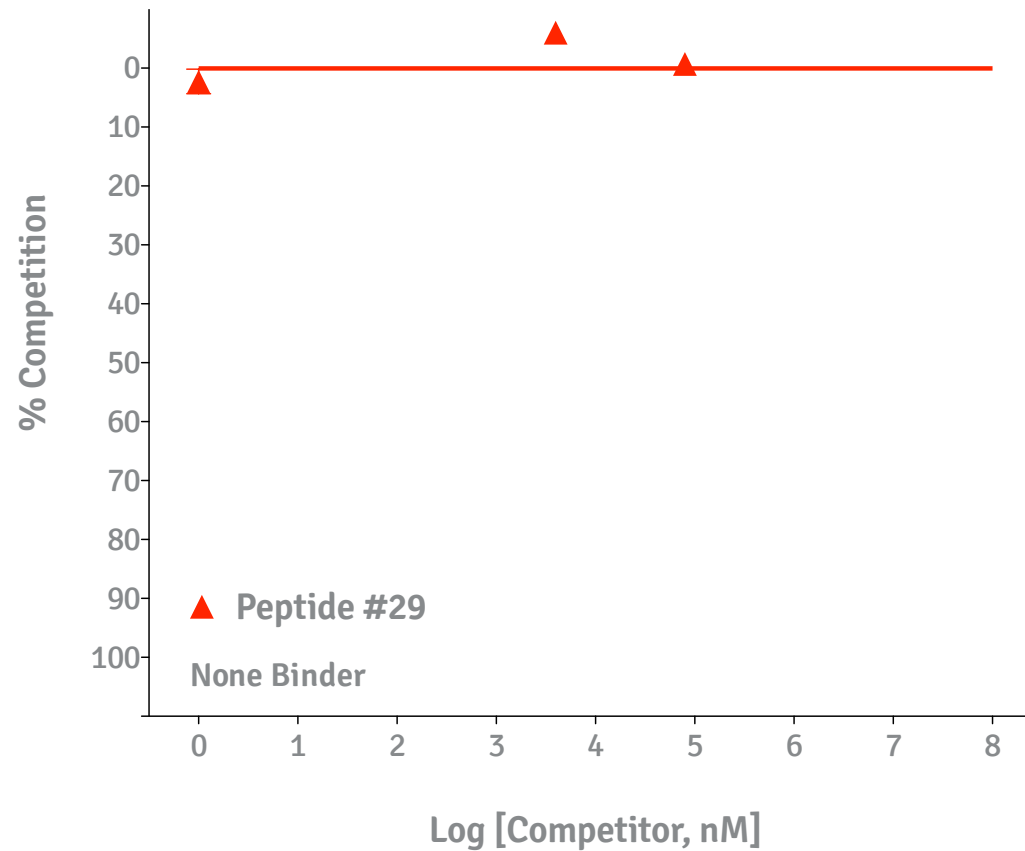
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CDCDCDCDCD
(PS000-28)



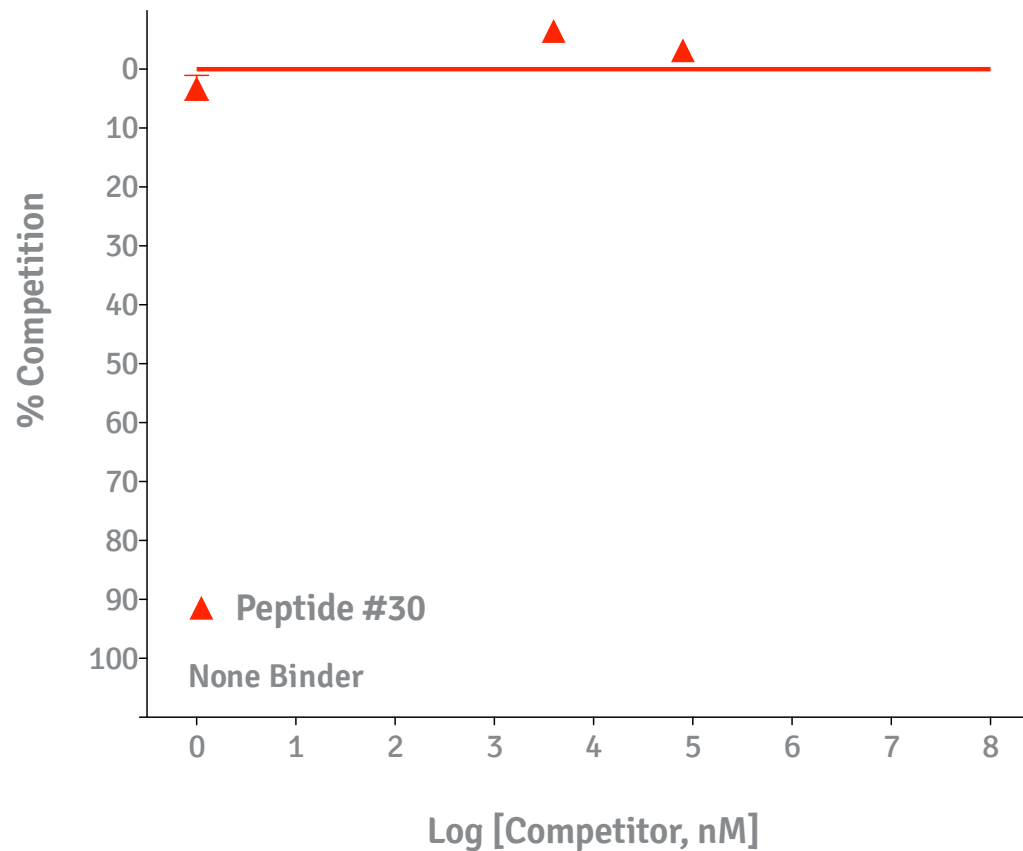
Peptide Epitope Screening Competition Assay PS-B*07:02

EFEFEFEFE
(PS000-29)



Peptide Epitope Screening Competition Assay PS-B*07:02

GHGHGHGHGH
(PS000-30)



If you have questions, need some additional explanation or different data formats, please don't hesitate contacting us:

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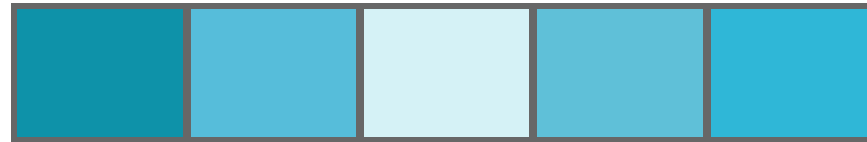
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0E92A9 56BDDA D5F2F6 5FC0D8 2FB7D7



676767 757879 808080 AF4EAF FFFFFFFF