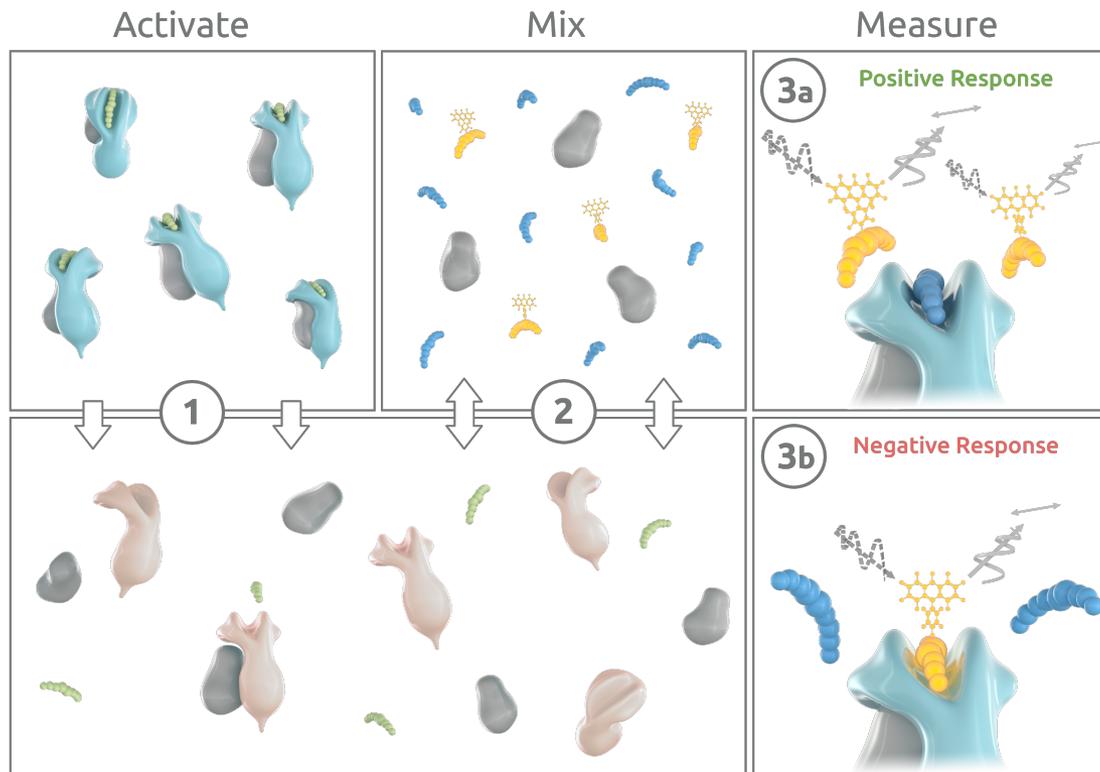


Peptide Epitope Validation Report



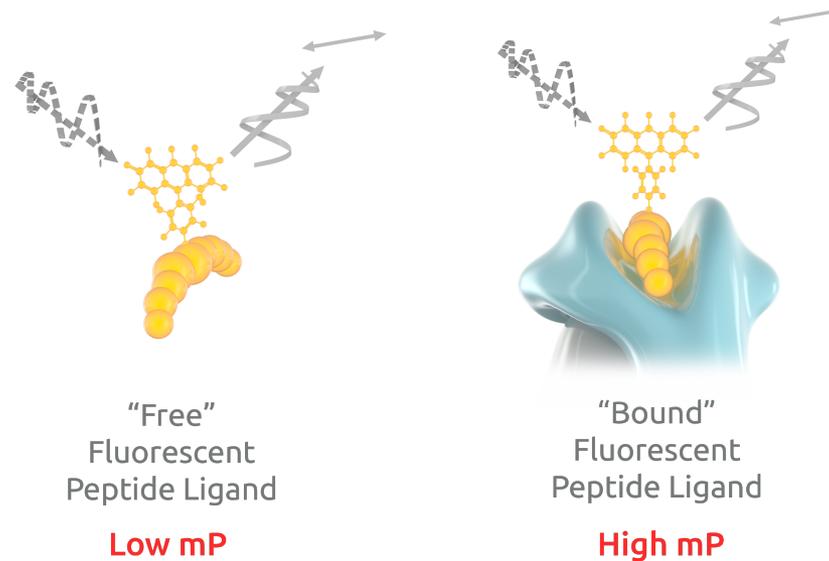
Report PV-A*02:01
March 23, 2020

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.

Within this study, inhibitory concentrations are determined by incubating sHLA with a labeled reference peptide in the presence of different concentrations of your competitor peptide. A calculated $\log C_{50}$ value is reported to you as measure of the effectiveness of the competing test peptide. Affinity categories shown will prioritize your $\log C_{50}$ values into high, medium, or low affinity binders.

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 $\log C_{50}$ 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	>	

Peptide Epitope Binding Results

MHC Peptide Sequence	Internal Name	HLA Allele	log[IC ₅₀]	Binding Category	R ²	Hill Slope
			log[(nM)]			
FLSELTQQL	MIF9	A*02:01	2.550	High Affinity	0.9995	-0.955
GILGFVFTL	INFM1	A*02:01	2.704	High Affinity	0.9959	-1.221
IMDQVPFSV	Gp100/IMD	A*02:01	2.753	High Affinity	0.9988	-0.795
YMDDVVLGA	HBV Pol (538)	A*02:01	2.868	High Affinity	0.9981	-1.256
FLPSDFFPSV	C1(A*0201)	A*02:01	2.906	High Affinity	0.9964	-1.261
LLGRNSFEV	p53	A*02:01	3.015	High Affinity	0.9987	-0.780
KLGEFYNQMM	C2(A*0201)	A*02:01	3.094	High Affinity	0.9999	-0.716
YLVSGVWI	HBV Core (118)	A*02:01	3.257	High Affinity	0.9865	-0.918
NLVPMVATV	CMVpp65	A*02:01	3.269	High Affinity	0.9967	-0.795
LLSSNLSWL	HBV Pol (407)	A*02:01	3.357	High Affinity	0.9965	-0.930
GLYSSTVPV	HBV Pol (61)	A*02:01	3.364	High Affinity	0.9991	-0.868
SLYNTVATL	HIV Gag (77)	A*02:01	3.635	High Affinity	0.9992	-1.124
GLSRYVARL	HBV Pol (455)	A*02:01	3.712	Medium Affinity	0.9971	-1.014
ILKEPVHGV	HIV Pol IV9	A*02:01	3.881	Medium Affinity	0.9988	-1.004
NLQSLTNLL	HBV Pol (399)	A*02:01	3.885	Medium Affinity	0.998	-0.8497
HLESFTAV	HBV Pol (551)	A*02:01	4.397	Medium Affinity	0.9989	-0.814
DLVHFASPL	HBV Pol (1420)	A*02:01	5.490	Low Affinity	0.996	-1.119

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	Performance Comments	Peptide Origin	Species	Sequence ID	Position
			log[[nM]]				(aa)							
FLSELTQQL	MIF9	A*02:01	2.550	High Affinity	0.9995	-0.955	9	PV173	09/11/12		Macrophage migration inhibitory factor (MIF)	Human	P14174	[19-27]
GILGFVFTL	INFM1	A*02:01	2.704	High Affinity	0.9959	-1.221	9	PV296	09/15/15		Matrix protein M1	Influenza A virus	P21429	[58-66]
IMDQVPFSV	Gp100/IMD	A*02:01	2.753	High Affinity	0.9988	-0.795	9	PV435	01/26/20		gp100	Human		[209-217]
YMDDVVLGA	HBV Pol (538)	A*02:01	2.868	High Affinity	0.9981	-1.256	9	PVH099	06/25/03		Pol	Hepatitis B virus (HBV)	P03156	[538-546]
FLPSDFPFSV	C1(A*0201)	A*02:01	2.906	High Affinity	0.9964	-1.261	10	PV219	04/11/13		Core	Hepatitis B virus (HBV)	P03146	[18-27]
LLGRNSFEV	p53	A*02:01	3.015	High Affinity	0.9987	-0.780	9	PV293	09/14/15		p53 (Tumor suppressor p53)	Human	P04637	[264-272]
KLGEFYNQMM	C2(A*0201)	A*02:01	3.094	High Affinity	0.9999	-0.716	10	PVH108	10/07/03		Nucleoprotein (NP)	Influenza B virus	P11102	[85-94]
YLVSGVWI	HBV Core (118)	A*02:01	3.257	High Affinity	0.9865	-0.918	9	PVH098	06/25/03		Core	Hepatitis B virus (HBV)	P03146	[118-126]
NLVPMTATV	CMVpp65	A*02:01	3.269	High Affinity	0.9967	-0.795	9	PV291	09/14/15		Tegument protein pp65	Human cytomegalovirus (CMV)		[495-503]
LLSNLSWL	HBV Pol (407)	A*02:01	3.357	High Affinity	0.9965	-0.930	9	PVH097	06/24/03		Pol	Hepatitis B virus (HBV)	P03156	[407-415]
GLYSSTVPV	HBV Pol (61)	A*02:01	3.364	High Affinity	0.9991	-0.868	9	PVH100_1	07/01/03		Pol	Hepatitis B virus (HBV)	P03156	[61-69]
SLYNTVATL	HIV Gag (77)	A*02:01	3.635	High Affinity	0.9992	-1.124	9	PVH070	12/16/02		Gag (p17)	Human Immunodeficiency Virus type 1 (HIV-1)	P05888	[77-85]
GLSRVVARL	HBV Pol (455)	A*02:01	3.712	Medium Affinity	0.9971	-1.014	9	PVH100_2	07/01/03		Pol	Hepatitis B virus (HBV)	P03156	[442-450]
ILKEPVHGV	HIV Pol IV9	A*02:01	3.881	Medium Affinity	0.9988	-1.004	9	PVH071	12/16/02		Pol	Human Immunodeficiency Virus type 1 (HIV-1)	P03368	[476-484]
NLQSLNLL	HBV Pol (399)	A*02:01	3.885	Medium Affinity	0.998	-0.8497	9	PVH099_2	06/25/03		Pol	Hepatitis B virus (HBV)	P03156	[400-408]
HLESFTAV	HBV Pol (551)	A*02:01	4.397	Medium Affinity	0.9989	-0.814	9	PVH100_3	07/01/03		Pol	Hepatitis B virus (HBV)	P03156	[551-559]
DLVHFASPL	HBV Pol (1420)	A*02:01	5.490	Low Affinity	0.996	-1.119	9	PVH088	05/22/03		Pol	Hepatitis B virus (HBV)	CAA46352	[817-825]

FULL DATA SET PROVIDED AS EXCEL WORKSHEET

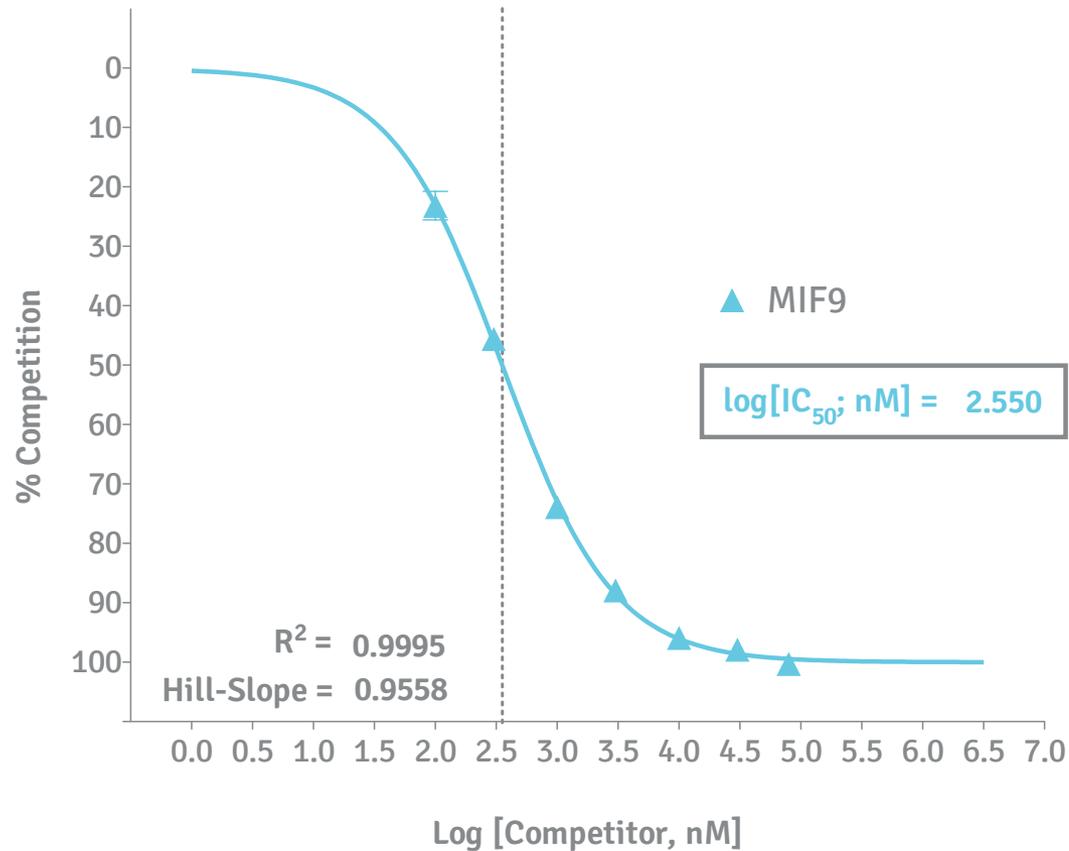
PV-Results.xlsx

IC₅₀ DATA COLLECTION



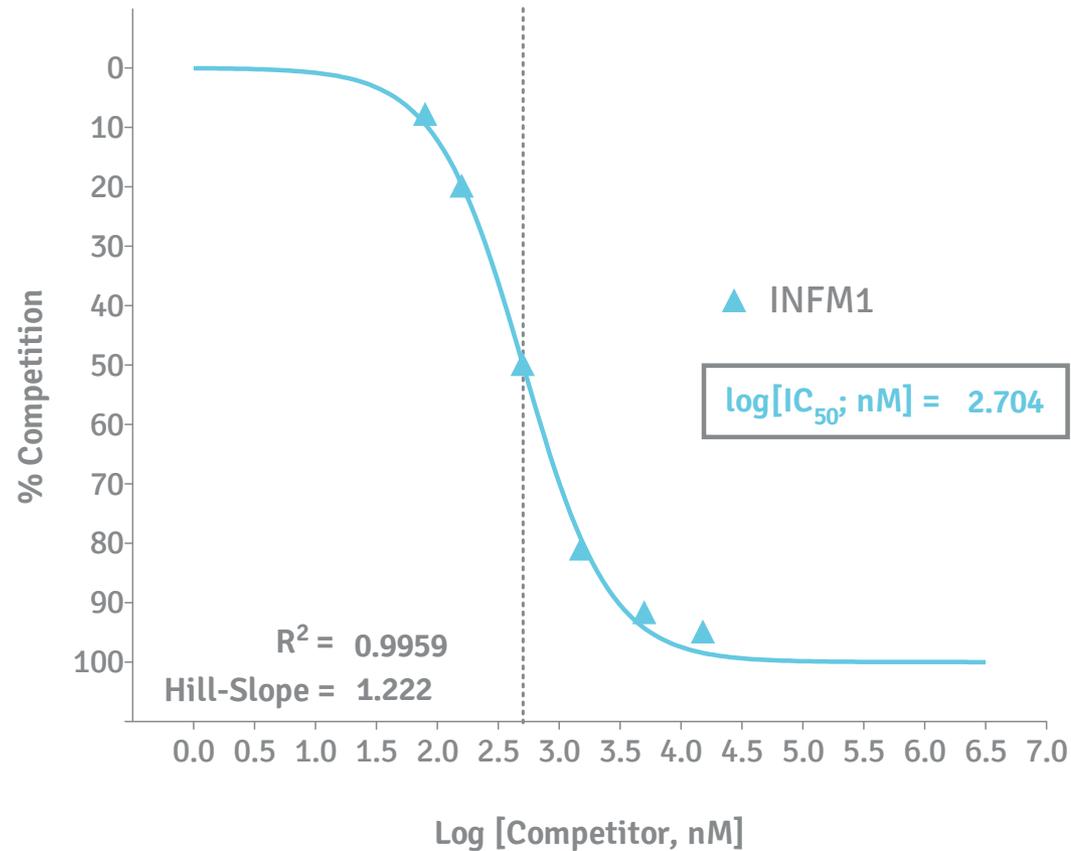
Peptide Epitope Validation Competition Assay PV-A*02:01

FLSELTQQL
(PV173-1)



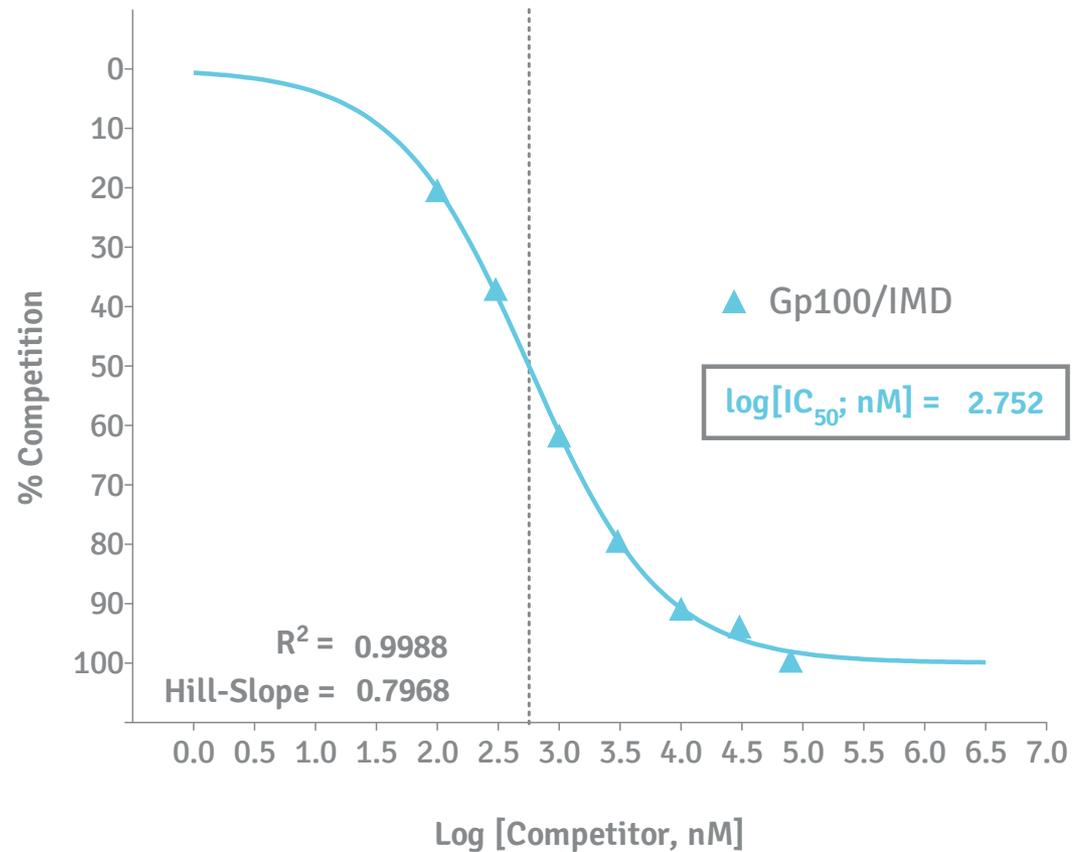
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GILGFVFTL
(PV296-1)



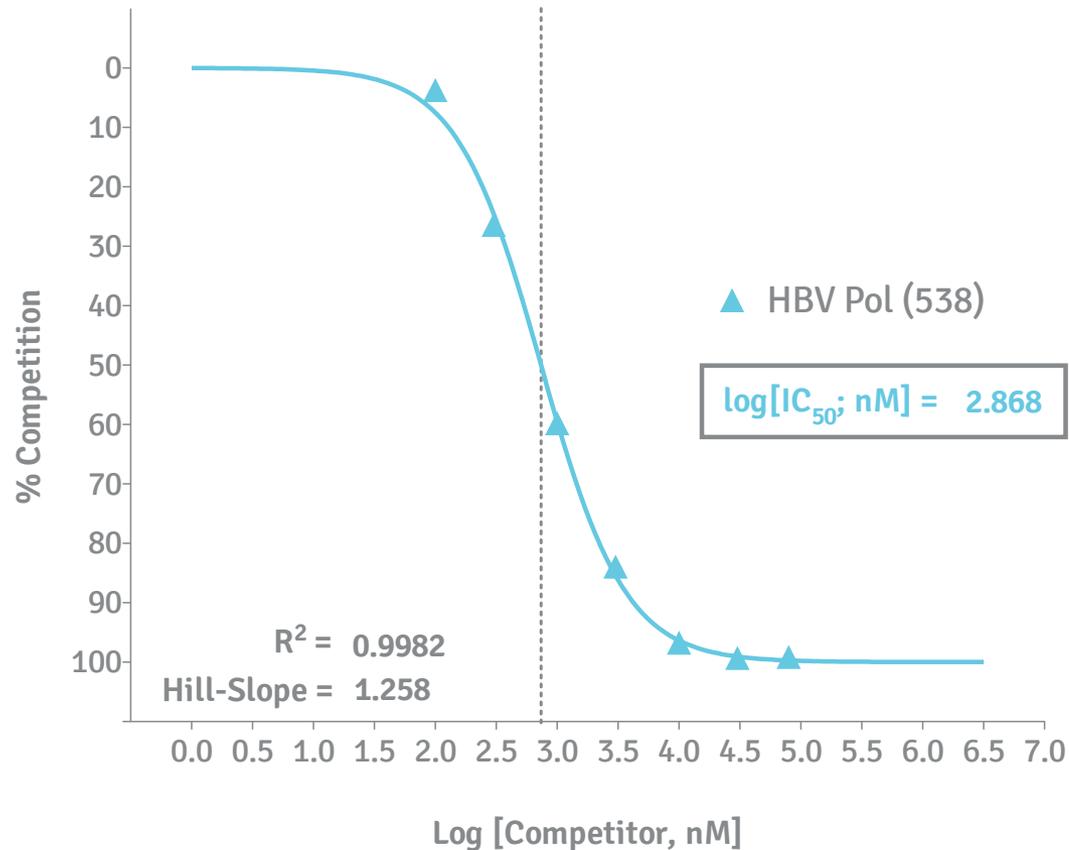
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IMDQVPFSV
(PV435-1)



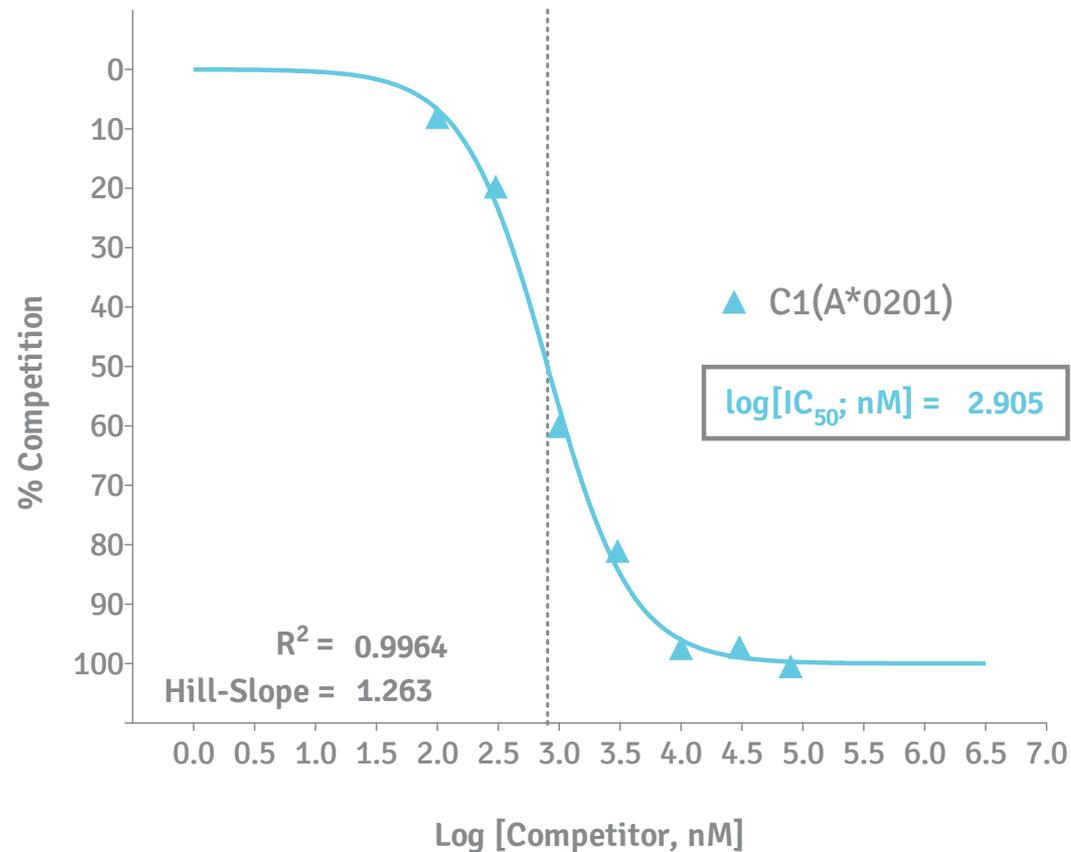
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YMDDVVLGA
(PVH099-1)



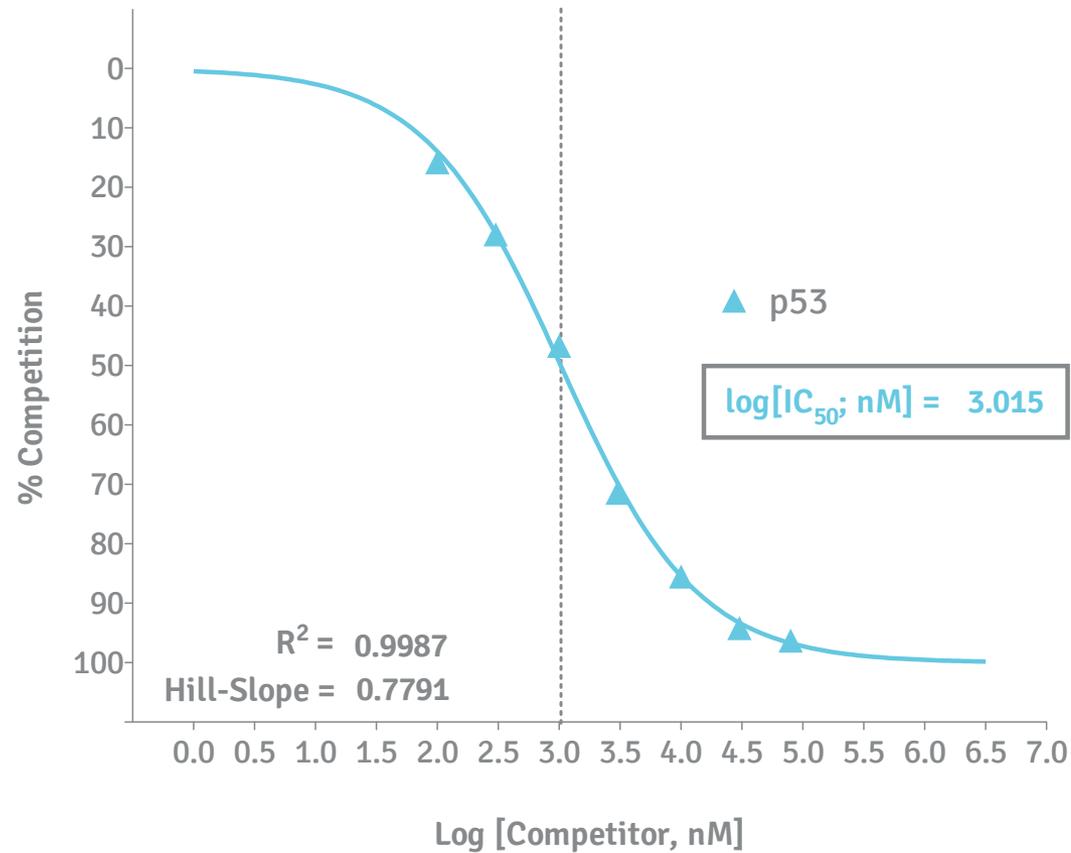
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FLPSDFFPSV
(PV219-1)



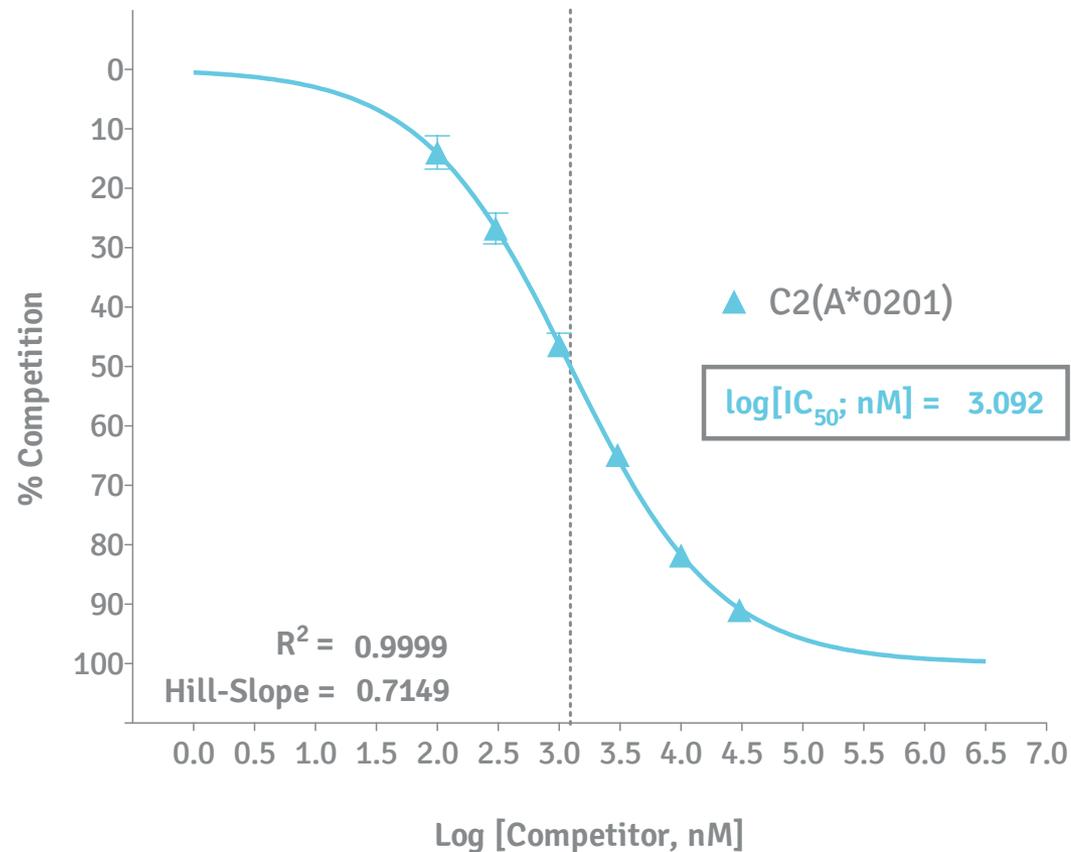
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LLGRNSFEV
(PV293-1)



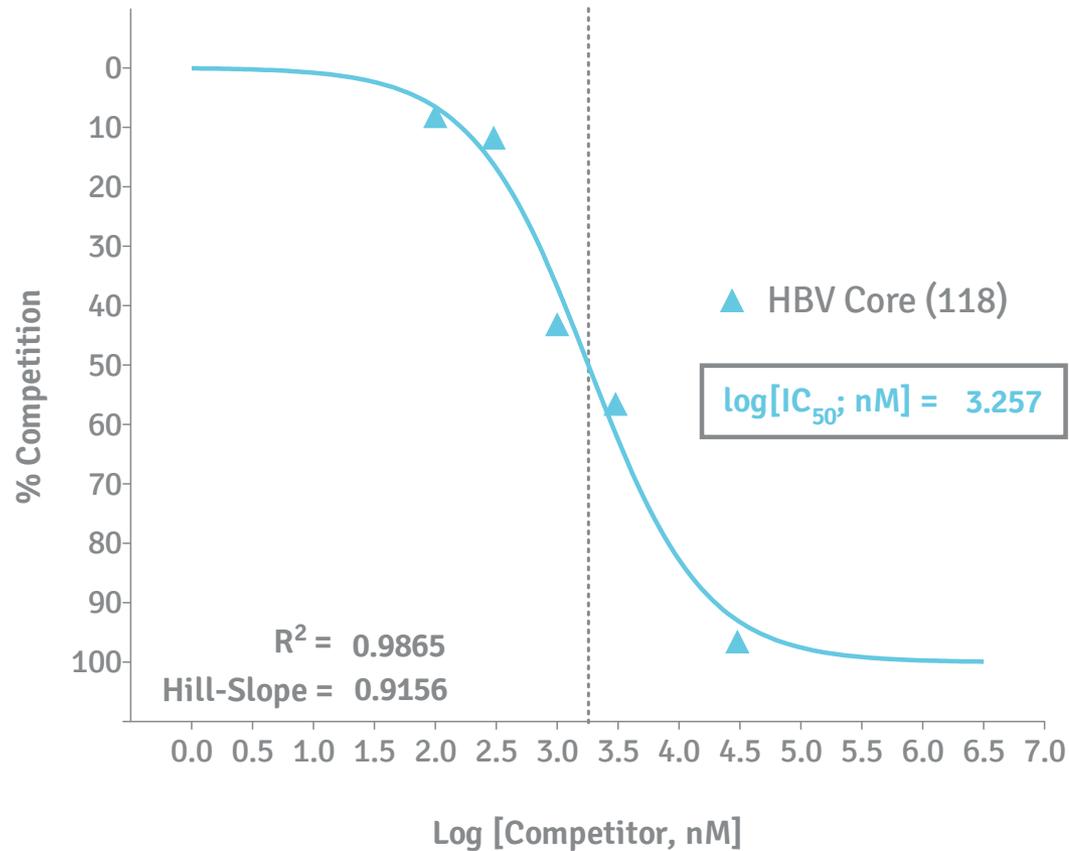
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KLGEFYNQMM
(PVH108-1)



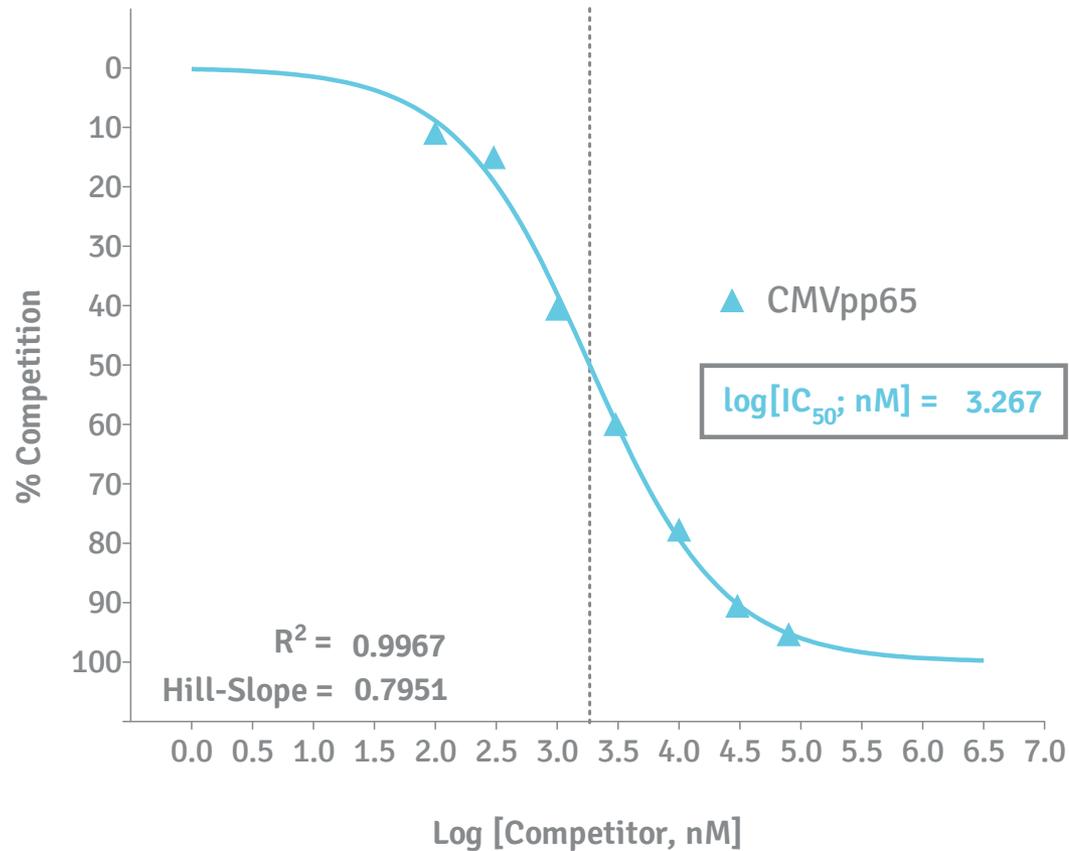
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YLVSFGVWI
(PVH098-1)



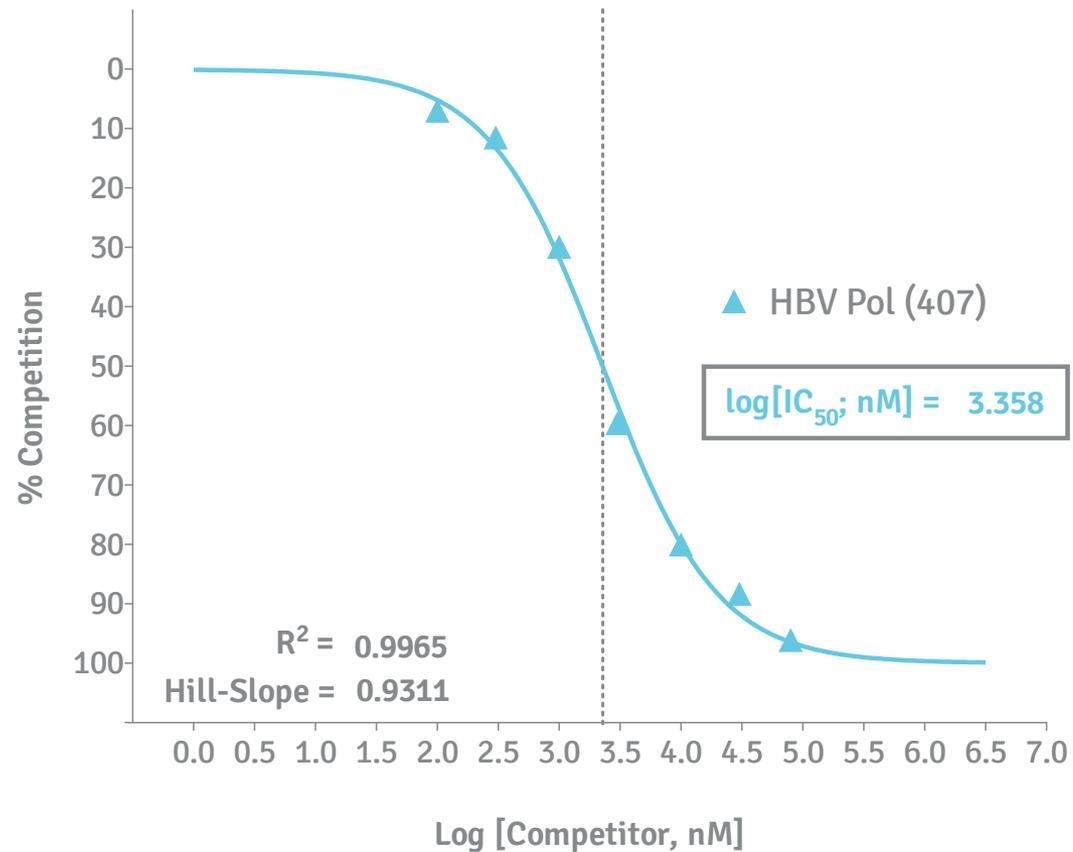
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NLVPMVATV
(PV291-1)



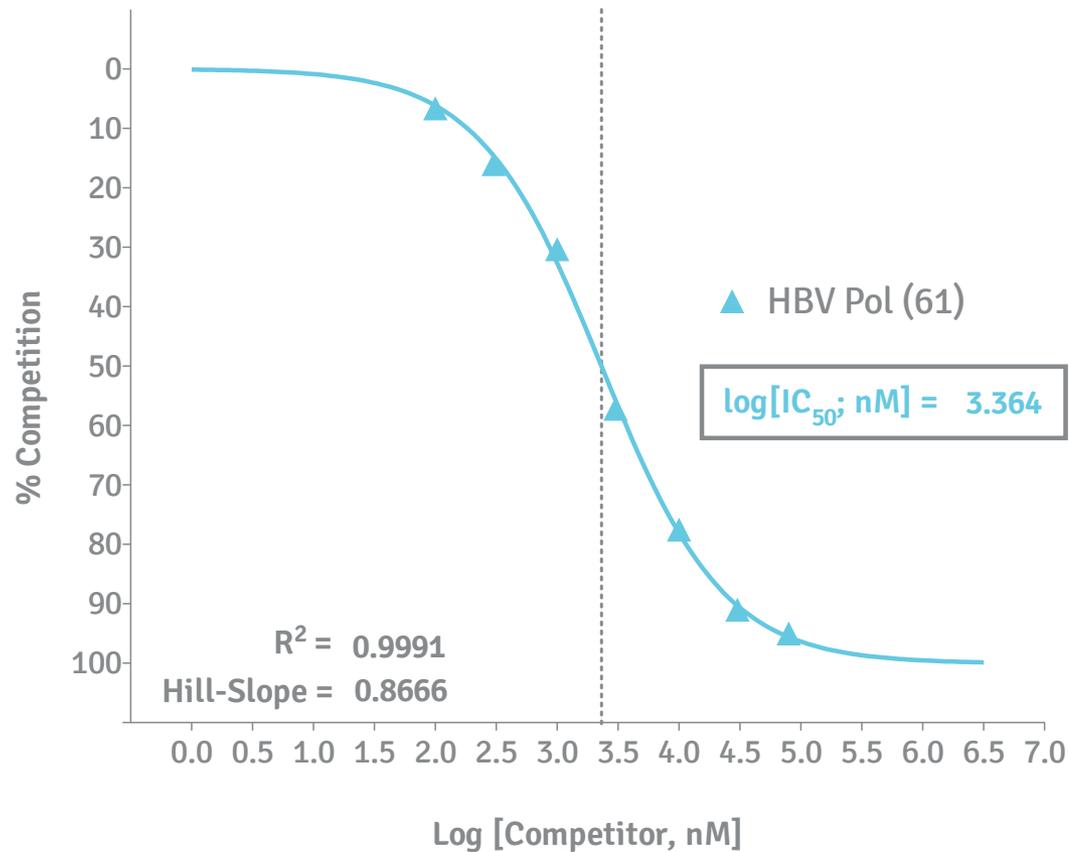
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LLSSNLSWL
(PVH097-1)



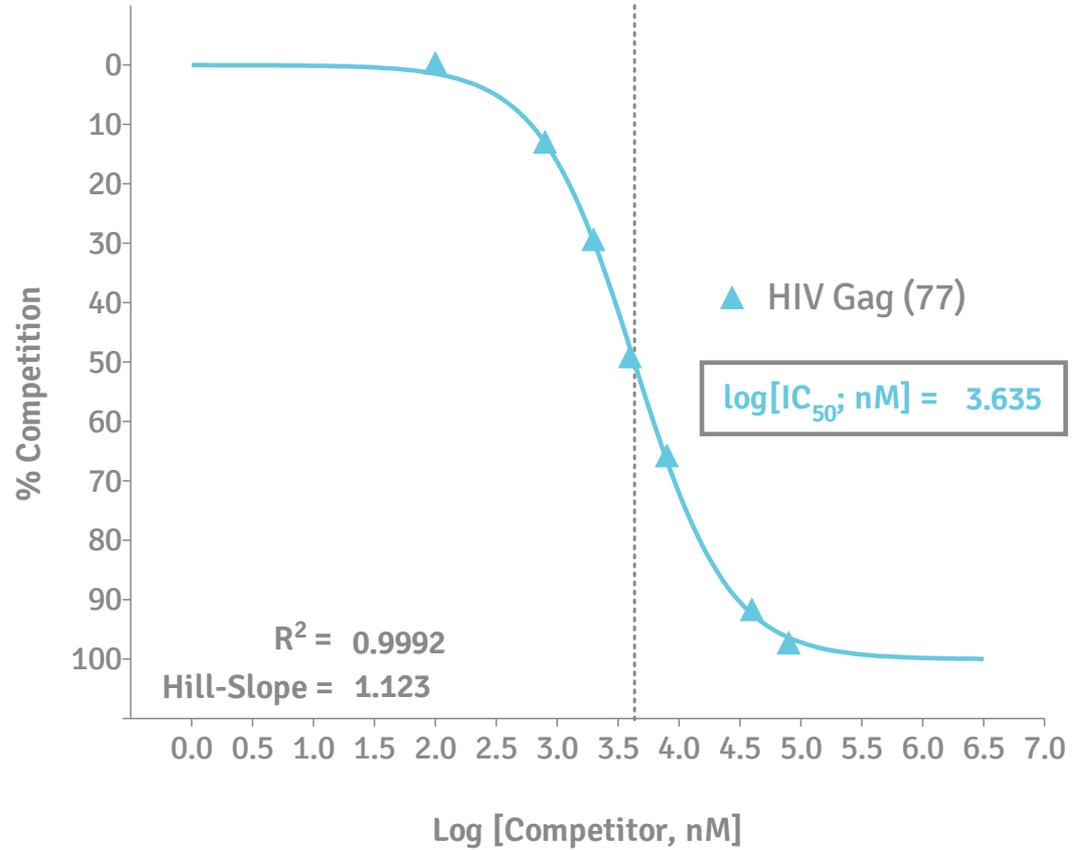
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GLYSSTVPV
(PVH100-1)



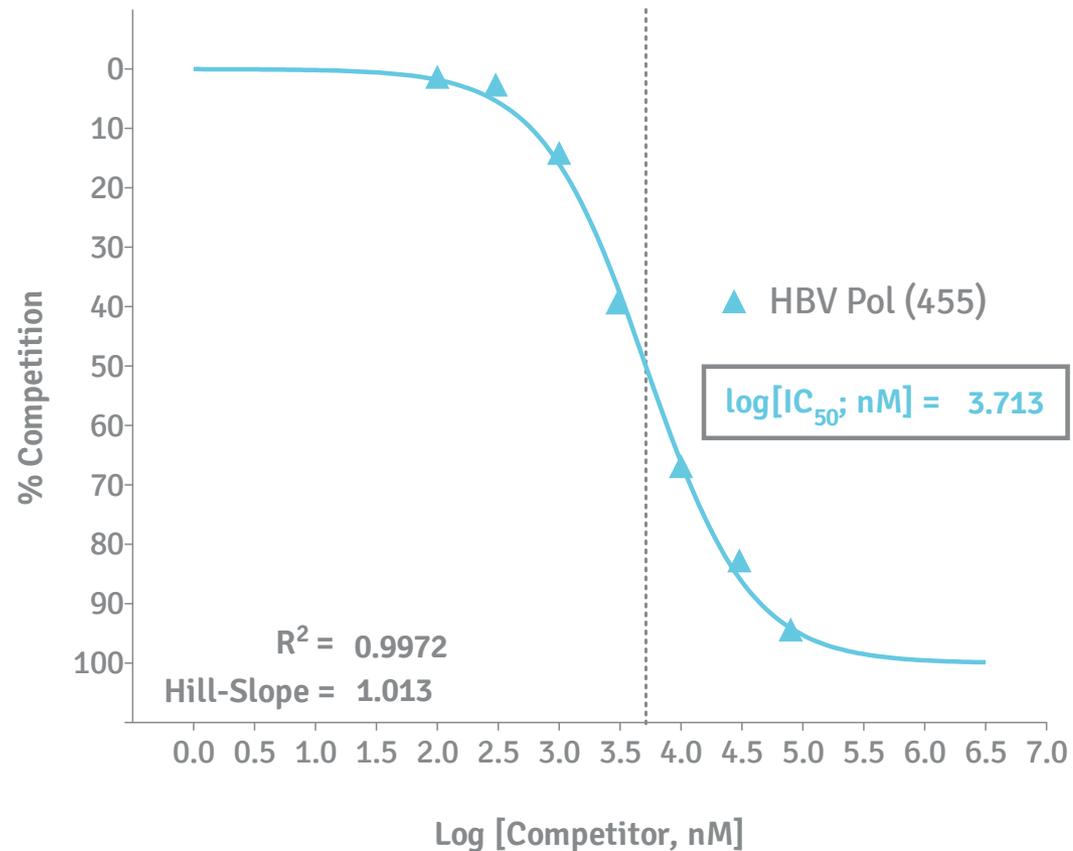
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SLYNTVATL
(PV070-1)



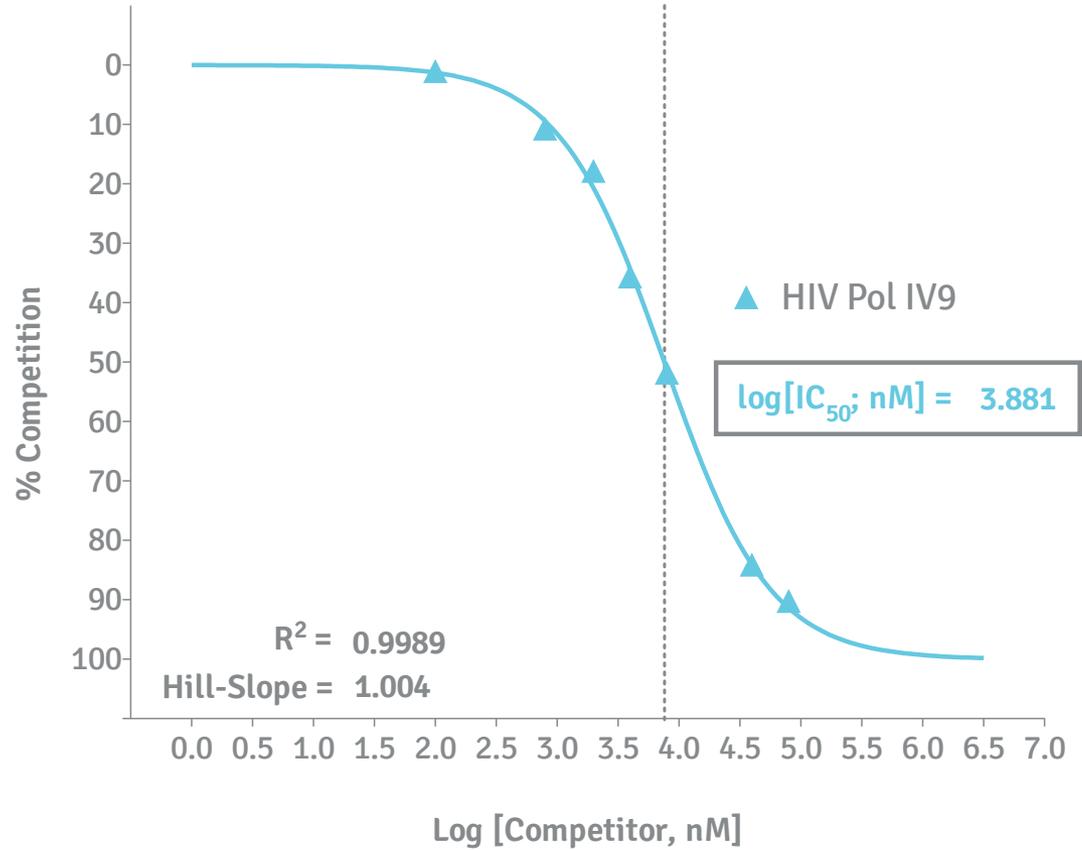
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GLSRYVARL
(PV100-2)



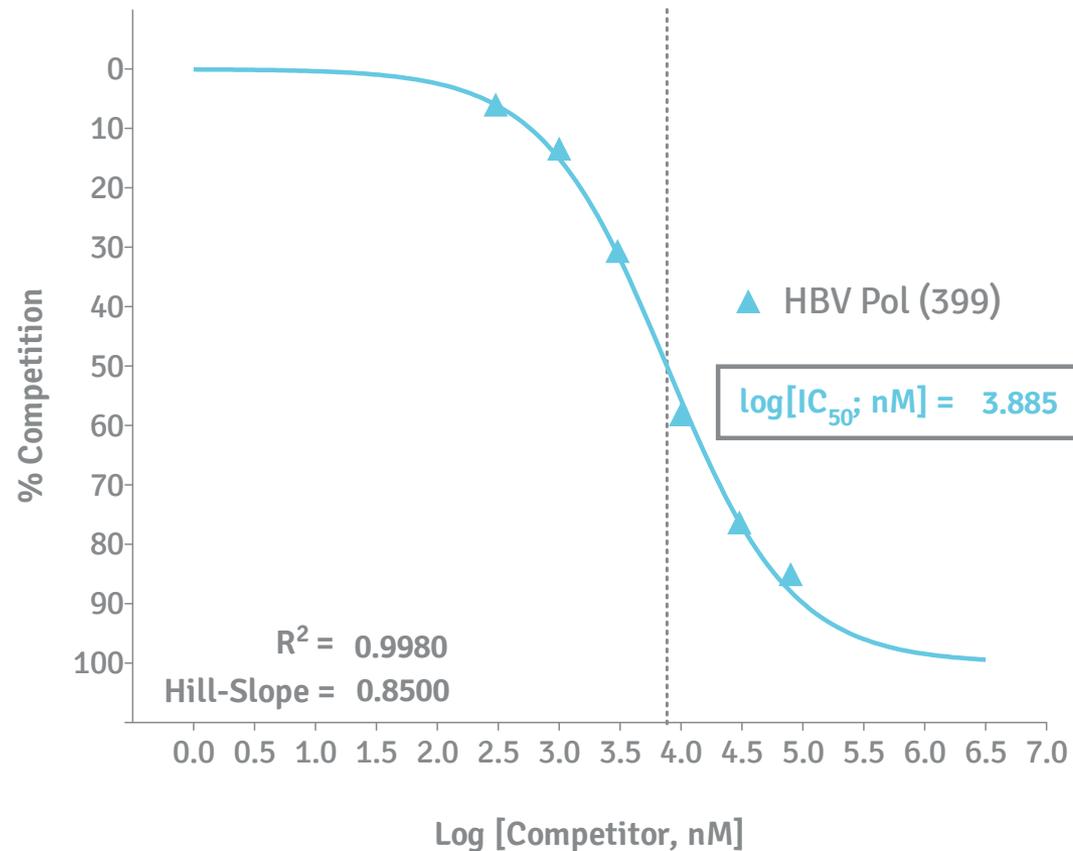
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ILKEPVHGV
(PVH071-1)



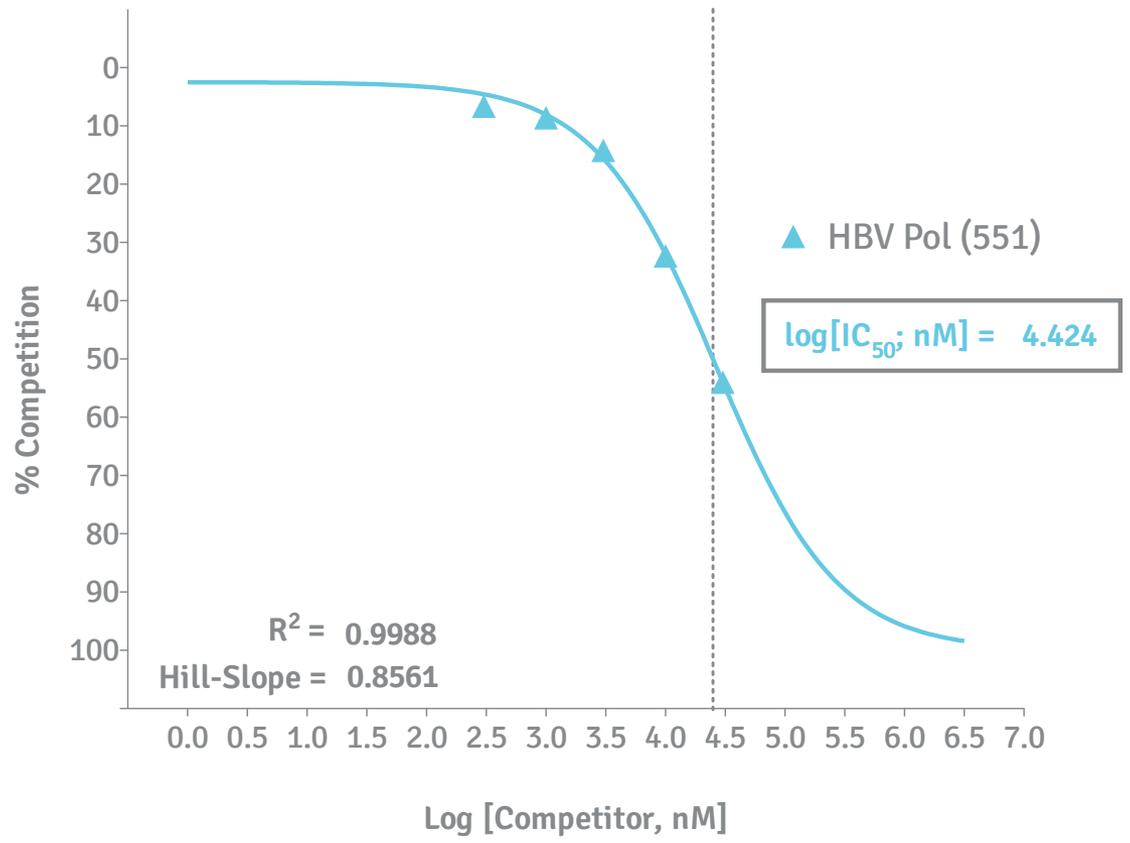
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NLQSLTNLL
(PVH099-2)



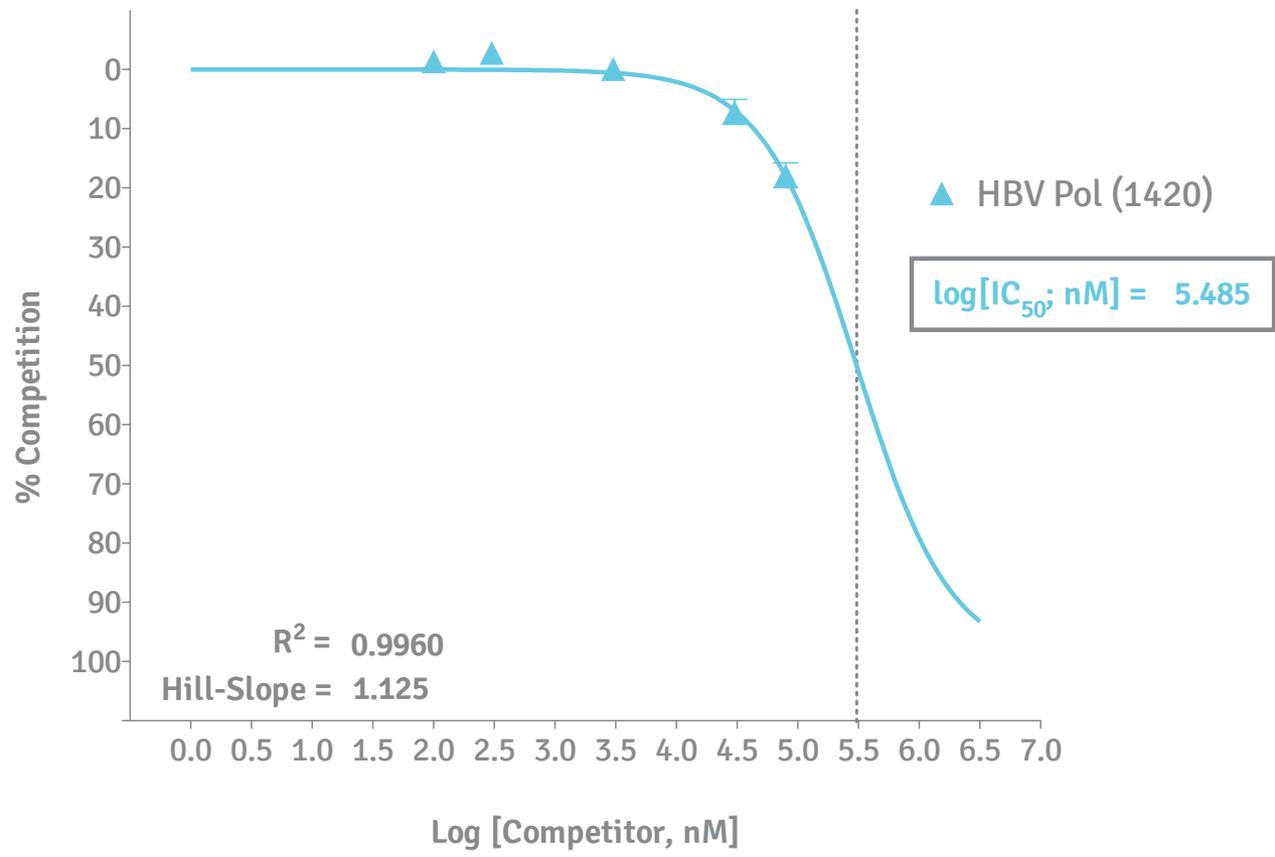
Peptide Epitope Validation Competition Assay PV-A*02:01

HLESLFTAV
(PV100-3)



Peptide Epitope Validation Competition Assay PV-A*02:01

DLVHFASPL
(PV088-1)



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

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