

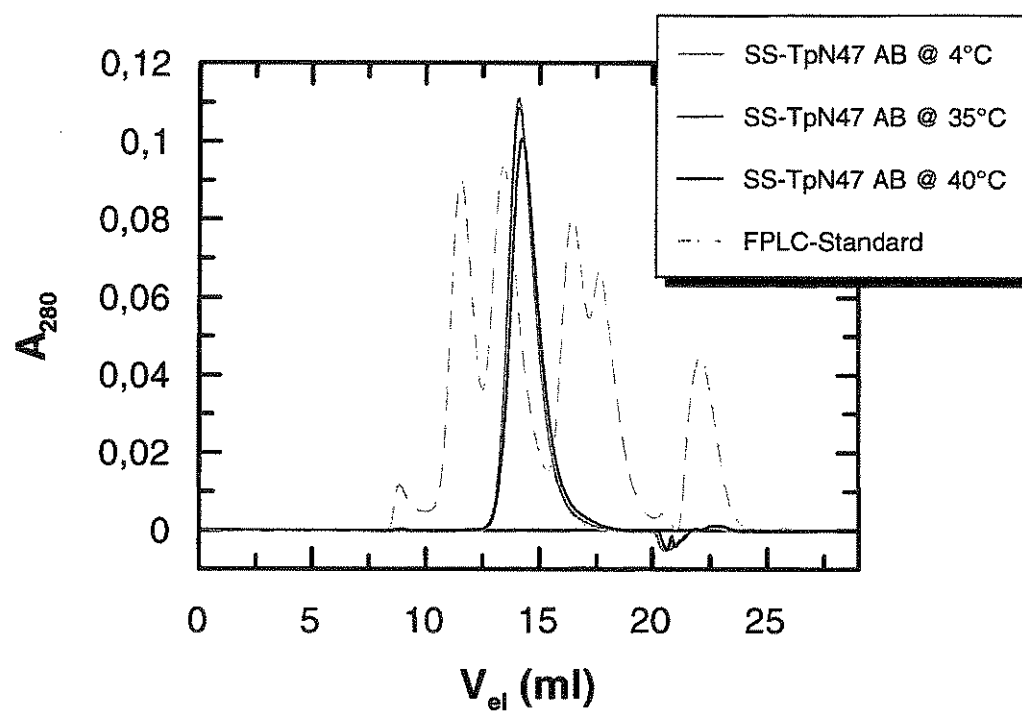


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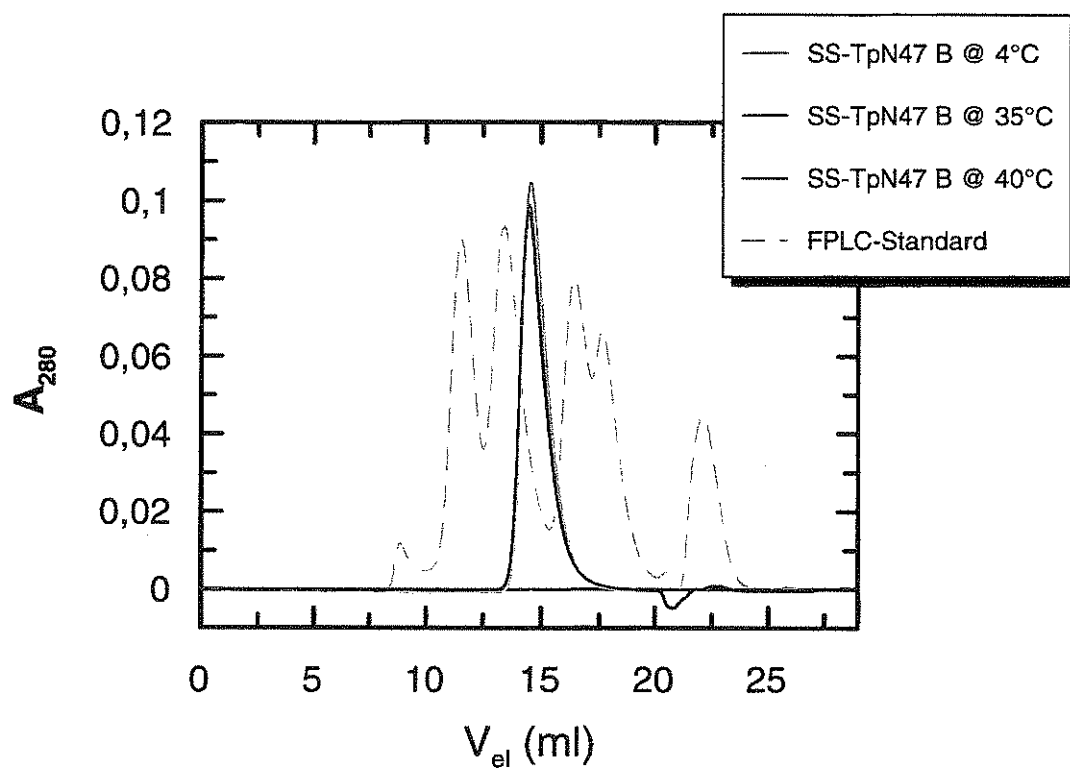


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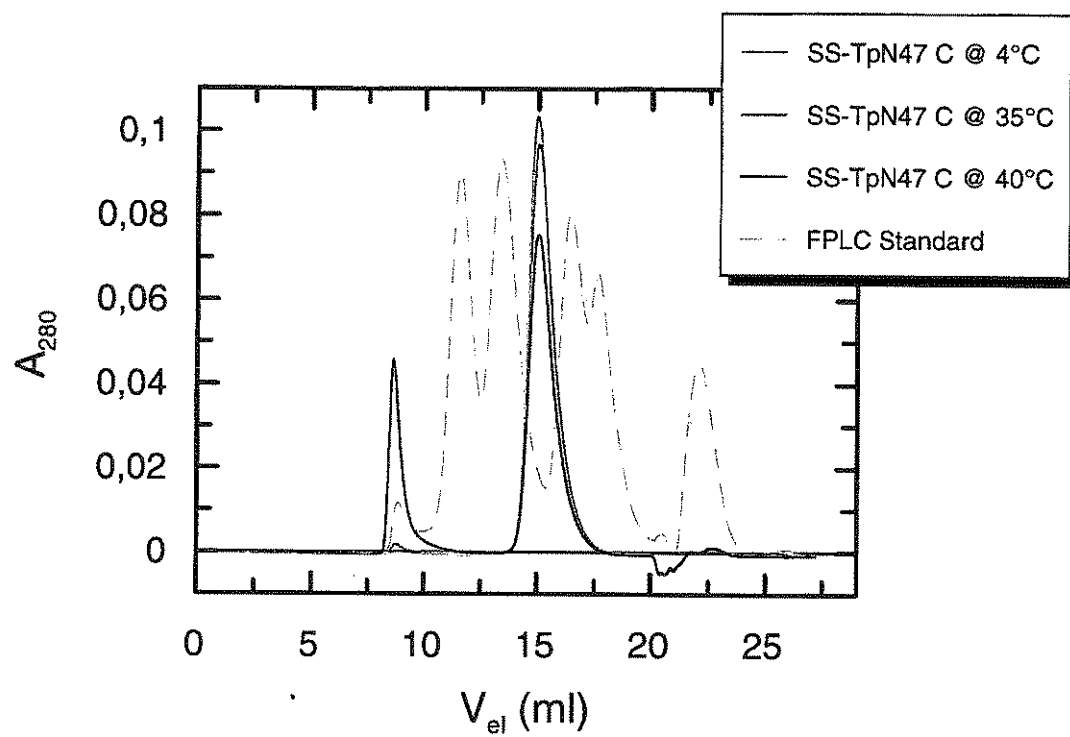


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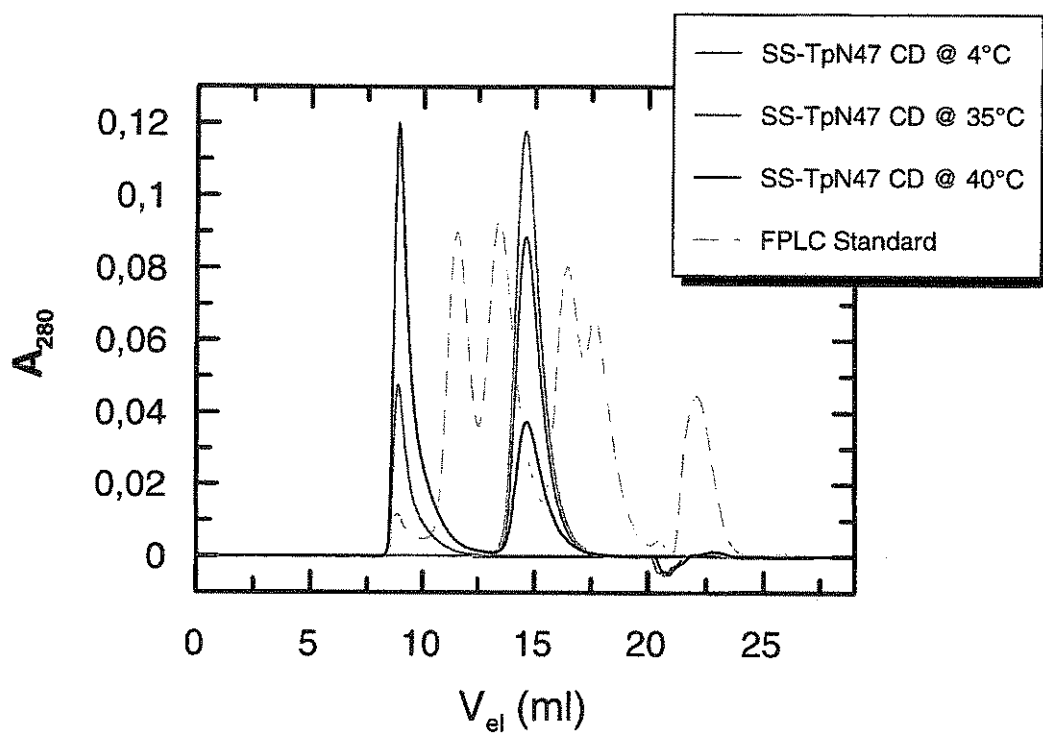


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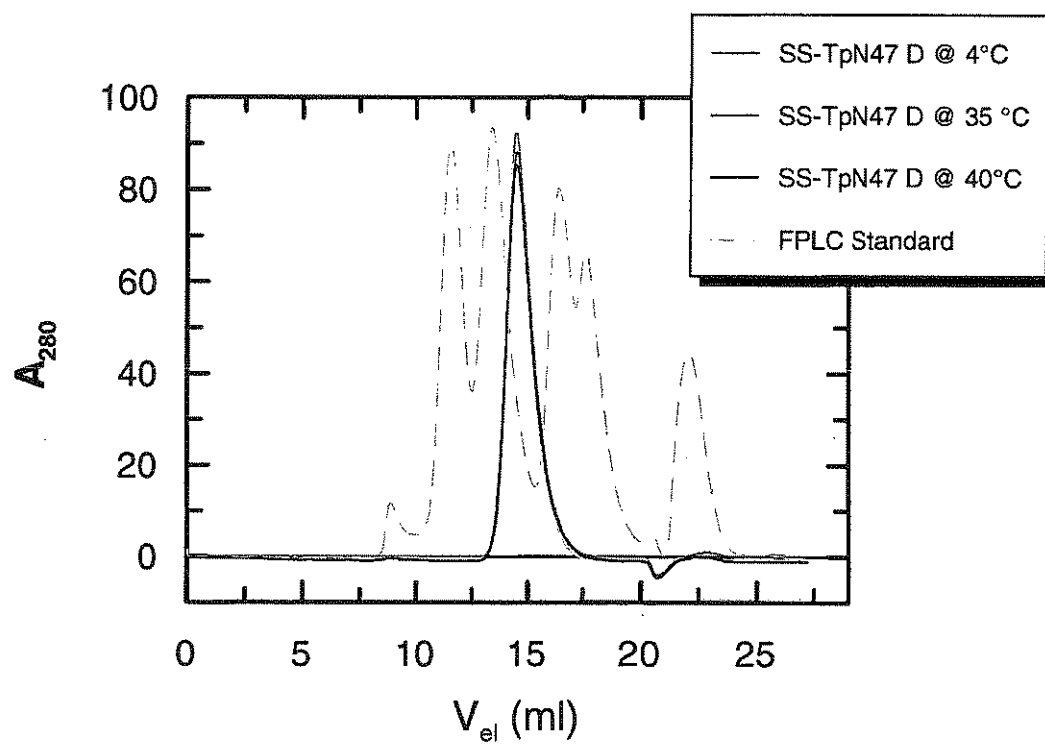


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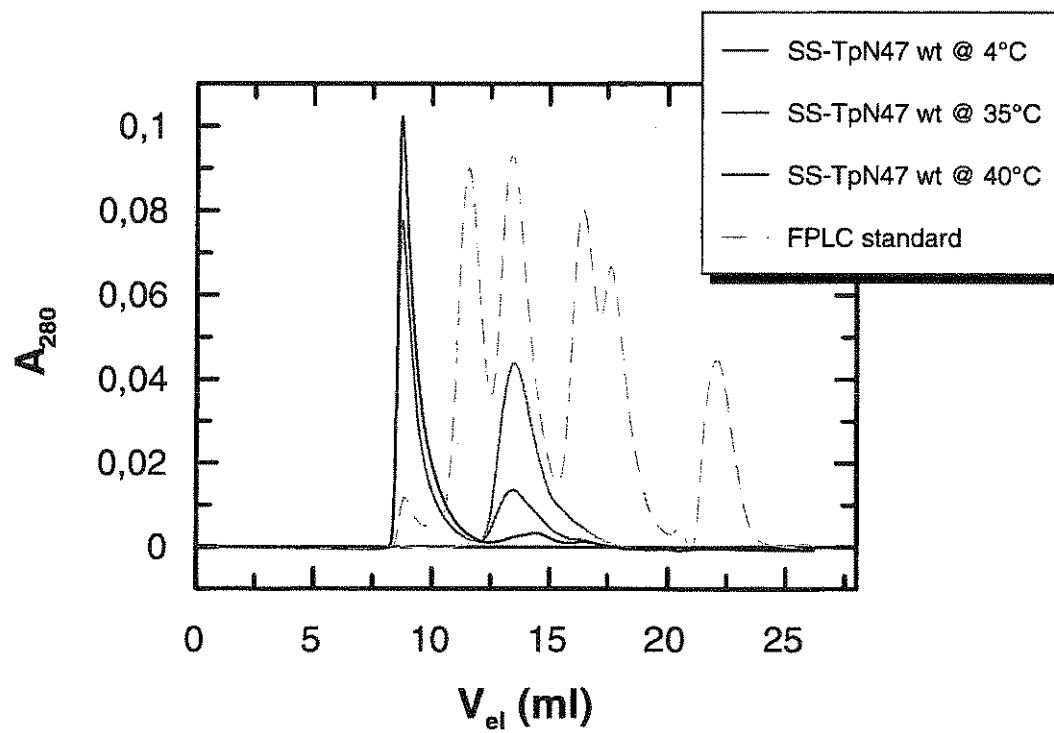


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Epitope Mapping of TpN47

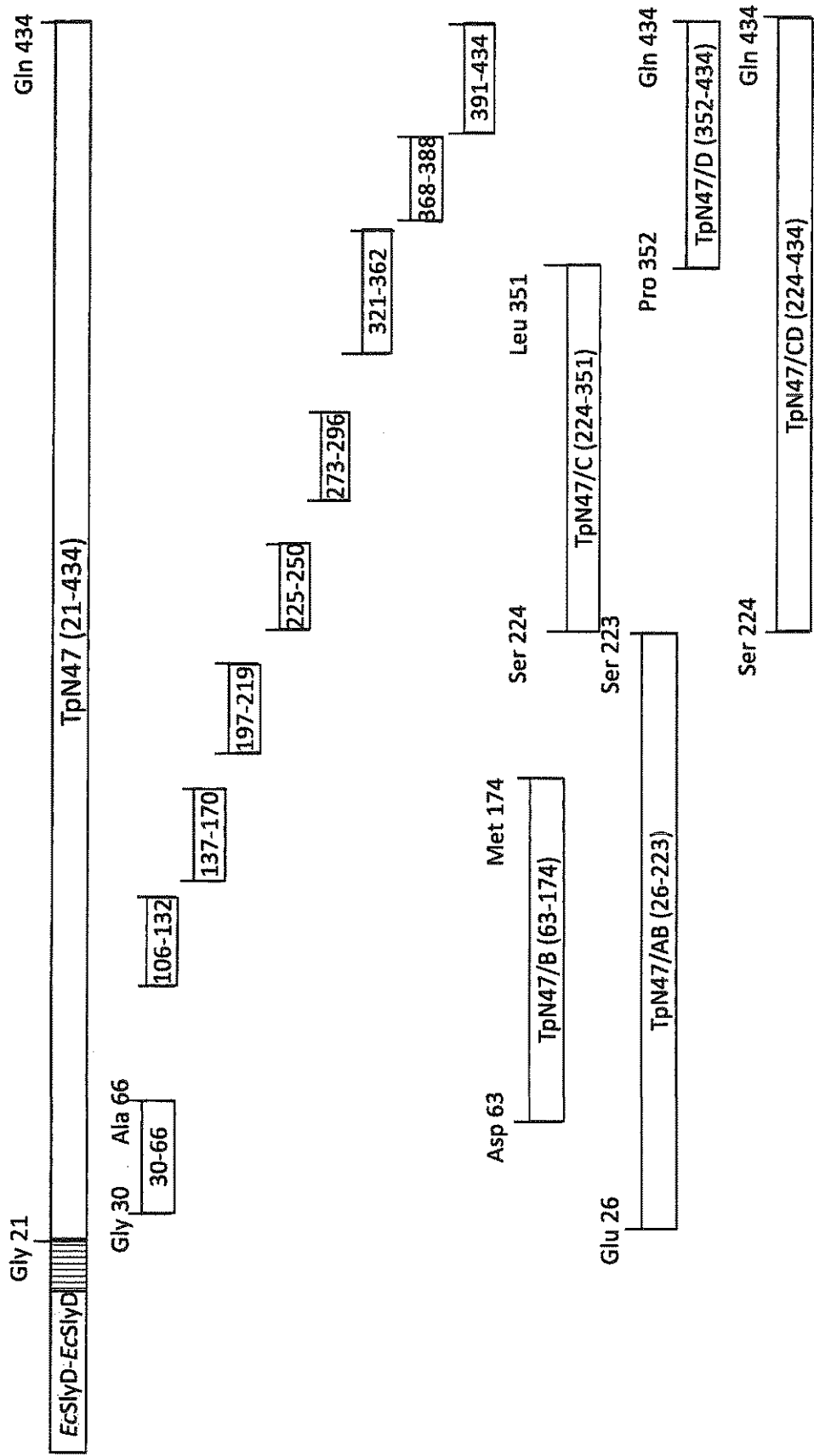


Figure 7/12

Table 2

Figure 8a/12

experiment	1		2		3		10	
R1	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47 (30-66)		TpN47 (106-132)		TpN47 (137-170)		TpN47 (21-434)	
label	Bi(DDS)		Bi(DDS)		Bi(DDS)		Bi(DDS)	
concentration Bi-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
R2	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47(30-66)		TpN47(106-132)		TpN47(137-170)		TpN47(21-434)	
label	BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)	
concentration Ru-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
sample	signal	signal-dynamics	signal	signal dyn.	signal	signal dyn.	signal	signal dyn.
average neg. sera	491	1.00	511	1.00	501	1.00	900	1.00
int. donor 4320	488	0.99	486	0.95	493	0.98	534	0.59
int. donor 4321	496	1.01	523	1.02	508	1.01	524	0.58
int. donor 4322	521	1.06	609	1.19	555	1.11	1,197	1.33
int. donor 4511	489	1.00	507	0.99	486	0.97	521	0.58
int. donor 4316	493	1.00	493	0.97	491	0.98	520	0.58
int. donor 4345	496	1.01	512	1.00	514	1.03	1,321	1.47
int. donor 4314	468	0.95	476	0.93	486	0.97	602	0.67
int. donor 4474	473	0.96	487	0.95	485	0.97	543	0.60
int. donor 4475	492	1.00	513	1.00	504	1.01	2,694	2.99
int. donor 4476	492	1.00	502	0.98	489	0.98	545	0.61
Syphilis IgG 1209A179	1,543	3.14	524	1.03	520	1.04	91,925	102.13
Syphilis IgG 1209A178	1,220	2.49	511	1.00	524	1.05	10,326	11.47
Syphilis IgG 1209A177	8,601	17.52	962	1.88	794	1.58	200,660	222.93
Syphilis IgG 1209A176	678	1.38	486	0.95	513	1.02	37,500	41.66
Syphilis IgG 1209A174	718	1.46	489	0.96	505	1.01	225,310	250.32
Syphilis IgM 1209A192	8,201	16.71	590	1.16	1,603	3.20	171,322	190.34
Syphilis IgM 1209A191	10,513	21.42	807	1.58	830	1.66	109,434	121.58
Syphilis IgM 1209A188	18,647	37.99	498	0.97	496	0.99	160,090	177.86
Syphilis IgM 1209A167	507	1.03	514	1.01	526	1.05	9,991	11.10
Syphilis IgM 1209A166	62,458	127.26	5,760	11.28	1,700	3.39	164,024	182.23

Figure 8a/12

Table 2

Figure 8b/12

experiment	4		5		6		10	
R1	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47 (197-219)		TpN47 (225-250)		TpN47 (273-296)		TpN47 (21-434)	
label	Bi(DDS)		Bi(DDS)		Bi(DDS)		Bi(DDS)	
concentration Bi-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
R2	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47(197-219)		TpN47(225-250)		TpN47(273-296)		TpN47(21-434)	
label	BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)	
concentration Ru-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
sample	signal	signal dyn.	signal	signal dyn.	signal	signal dyn.	signal	signal dyn.
average neg. sera	503	1.00	497	1.00	506	1.00	900	1.00
int. donor 4320	496	0.99	494	0.99	504	1.00	534	0.59
int. donor 4321	507	1.01	500	1.01	524	1.04	524	0.58
int. donor 4322	545	1.08	515	1.04	558	1.10	1,197	1.33
int. donor 4511	497	0.99	493	0.99	512	1.01	521	0.58
int. donor 4316	487	0.97	496	1.00	487	0.96	520	0.58
int. donor 4345	516	1.03	511	1.03	512	1.01	1,321	1.47
int. donor 4314	477	0.95	479	0.96	477	0.94	602	0.67
int. donor 4474	483	0.96	498	1.00	497	0.98	543	0.60
int. donor 4475	521	1.04	491	0.99	497	0.98	2,694	2.99
int. donor 4476	502	1.00	492	0.99	488	0.97	545	0.61
Syphilis IgG 1209A179	508	1.01	510	1.03	515	1.02	91,925	102.13
Syphilis IgG 1209A178	506	1.01	488	0.98	507	1.00	10,326	11.47
Syphilis IgG 1209A177	631	1.25	556	1.12	640	1.27	200,660	222.93
Syphilis IgG 1209A176	503	1.00	495	1.00	508	1.00	37,500	41.66
Syphilis IgG 1209A174	486	0.97	493	0.99	487	0.96	225,310	250.32
Syphilis IgM 1209A192	533	1.06	505	1.02	516	1.02	171,322	190.34
Syphilis IgM 1209A191	643	1.28	616	1.24	564	1.12	109,434	121.58
Syphilis IgM 1209A188	495	0.98	468	0.94	492	0.97	160,090	177.86
Syphilis IgM 1209A167	523	1.04	507	1.02	515	1.02	9,991	11.10
Syphilis IgM 1209A166	985	1.96	769	1.55	1,133	2.24	164,024	182.23

Figure 8b/12

Table 2

Figure 8c/12

experiment	7		8		9		10	
R1	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47 (321-362)		TpN47 (368-388)		TpN47 (391-434)		TpN47 (21-434)	
label	Bi(DDS)		Bi(DDS)		Bi(DDS)		Bi(DDS)	
concentration Bi-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
R2	rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -		rec. <i>EcSlyD</i> -	
	TpN47(321- 362)		TpN47(368- 388)		TpN47(391- 434)		TpN47(21-434)	
label	BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)		BPRu(SK(2)DSS)	
concentration Ru-conjugate	70 ng/ml		70 ng/ml		70 ng/ml		70 ng/ml	
sample	signal	signal dyn.	signal	signal dyn.	signal	signal dyn.	signal	signal dyn.
average neg. sera	498	1.00	489	1.00	518	1.00	900	1.00
int. donor 4320	489	0.98	482	0.99	502	0.97	534	0.59
int. donor 4321	503	1.01	495	1.01	521	1.01	524	0.58
int. donor 4322	553	1.11	518	1.06	577	1.11	1,197	1.33
int. donor 4511	488	0.98	478	0.98	531	1.02	521	0.58
int. donor 4316	476	0.96	487	1.00	503	0.97	520	0.58
int. donor 4345	500	1.01	496	1.01	530	1.02	1,321	1.47
int. donor 4314	478	0.96	473	0.97	485	0.94	602	0.67
int. donor 4474	491	0.99	490	1.00	514	0.99	543	0.60
int. donor 4475	495	0.99	478	0.98	503	0.97	2,694	2.99
int. donor 4476	502	1.01	492	1.01	517	1.00	545	0.61
Syphilis IgG 1209A179	508	1.02	492	1.01	515	0.99	91,925	102.13
Syphilis IgG 1209A178	487	0.98	494	1.01	523	1.01	10,326	11.47
Syphilis IgG 1209A177	635	1.28	556	1.14	845	1.63	200,660	222.93
Syphilis IgG 1209A176	507	1.02	492	1.01	528	1.02	37,500	41.66
Syphilis IgG 1209A174	490	0.98	468	0.96	500	0.96	225,310	250.32
Syphilis IgM 1209A192	498	1.00	485	0.99	576	1.11	171,322	190.34
Syphilis IgM 1209A191	480	0.96	589	1.20	688	1.33	109,434	121.58
Syphilis IgM 1209A188	482	0.97	483	0.99	509	0.98	160,090	177.86
Syphilis IgM 1209A167	506	1.02	514	1.05	531	1.02	9,991	11.10
Syphilis IgM 1209A166	1,282	2.58	720	1.47	1,656	3.20	164,024	182.23

Figure 8c/12

Figure 9/12

Table 3

R1: EcSlyD-EcSlyD- [TpN47 domain]-Biotin	AB		B		C		D		CD		ABCD	
	sig	signal	sig	signal	sig	signal	sig	signal	sig	signal	sig	signal
R2: EcSlyD-EcSlyD- [TpN47 domain]-Ru	[cts]	dyn.	[cts]	dyn.	[cts]	dyn.	[cts]	dyn.	[cts]	dyn.	[cts]	dyn.
conc. Bi/Ru conjugate	70 ng/ml											
sample	70 ng/ml											
average neg. sera	454		467		594		472		541		515	
int. donor 4320	453	1.00	476	1.02	580	0.98	477	1.01	529	0.98	518	1.01
int. donor 4321	463	1.02	476	1.02	608	1.02	482	1.02	551	1.02	528	1.02
int. donor 4322	468	1.03	476	1.02	623	1.05	481	1.02	557	1.03	525	1.02
int. donor 4475	449	0.99	467	1.00	588	0.99	471	1.00	542	1.00	519	1.01
int. donor 4474	444	0.98	465	1.00	591	1.00	459	0.97	551	1.02	519	1.01
int. donor 4345	465	1.02	455	0.97	574	0.97	509	1.08	538	0.99	508	0.99
int. donor 4314	449	0.99	455	0.97	596	1.00	448	0.95	527	0.98	494	0.96
int. donor 4511	452	1.00	466	1.00	594	1.00	470	1.00	537	0.99	507	0.99
int. donor 4316	447	0.98	460	0.98	588	0.99	456	0.97	534	0.99	503	0.98
int. donor 4476	453	1.00	478	1.02	596	1.00	462	0.98	540	1.00	527	1.02
Syphilis IgG 1209A179	12,359	27.21	7,615	16.30	596	1.00	11,616	24.63	15,227	28.17	29,592	57.49
Syphilis IgG 1209A178	2,356	5.19	1,393	2.98	596	1.00	850	1.80	1,087	2.01	3,260	6.33
Syphilis IgG 1209A177	60,282	132.72	41,889	89.63	563	0.95	13,094	27.77	17,530	32.43	94,880	184.32
Syphilis IgG 1209A176	8,447	18.60	6,204	13.27	607	1.02	1,773	3.76	2,014	3.73	11,894	23.11
Syphilis IgG 1209A174	44,791	98.61	35,280	75.49	595	1.00	37,192	78.87	48,197	89.15	104,758	203.51
Syphilis IgM 1209A192	75,638	166.53	31,696	67.82	881	1.48	13,250	28.10	26,668	49.33	124,027	240.94
Syphilis IgM 1209A191	121,724	267.99	77,708	166.28	608	1.02	38,260	81.13	58,394	108.01	150,789	292.93
Syphilis IgM 1209A188	45,905	101.07	23,721	50.76	582	0.98	1,569	3.33	2,895	5.36	62,311	121.05
Syphilis IgM 1209A167	1,216	2.68	829	1.77	613	1.03	507	1.08	674	1.25	1,887	3.67
Syphilis IgM 1209A166	162,863	358.57	81,235	173.82	644	1.08	38,163	80.93	58,444	108.11	178,318	346.41

Figure 9/12

Table 4

Figure 10/12

R1	<i>EcSlyD-EcSlyD-TpN47[AB] -Bi</i>				<i>EcSlyD-EcSlyD-TpN47 [B] -Bi</i>			
	TpN47 (26-223)				TpN47 (63-174)			
	70ng/ml				70ng/ml			
	72h 4°C	72h 42°C	signal recovery		72h 4°C	72h 42°C	signal recovery	
R2	<i>EcSlyD-EcSlyD-TpN47 [AB] -Ru</i>				<i>EcSlyD-EcSlyD-TpN47 [B] -Ru</i>			
	TpN47 (26-223)				TpN47 (63-174)			
	70ng/ml				70ng/ml			
	72h 4°C	72h 42°C	signal recovery		72h 4°C	72h 42°C	signal recovery	
Thermal stress	cts	SD	cts	SD	cts	SD	cts	SD
	2,859	6.37	2,114	4.67	1,571	3.33	1,475	3.15
	2,892	6.44	2,661	5.88	1,862	3.94	1,768	3.78
	47,659	106	42,159	93.1	45,895	97.2	42,945	91.8
BM 200830	37,619	83.8	33,297	73.5	36,750	77.8	33,793	72.2
BM 200831	17,346	38.7	15,372	33.9	10,361	21.9	9,617	20.5
BM 202017	18,178	40.5	15,656	34.6	10,606	22.5	9,763	20.9
BM 202018	23,179	51.6	20,424	45.1	13,492	28.6	12,700	27.1
BM 206252	447	1.00	447	0.987	478	1.01	468	0.999
BM 206253	450	1.00	467	1.03	462	0.979	463	0.990
BM 206254	449	1.00	460	1.02	482	1.02	471	1.01
Trina #097	450	1.00	437	0.966	466	0.987	470	1.01
Trina #098			453		472		468	
Trina #099								
Trina #100								
average negative	449							

Figure 10/12

Table 5

Figure 11/12

R1	<i>EcSlyD-EcSlyD-TpN47[C] -Bi</i>				<i>EcSlyD-EcSlyD-TpN47[D] -Bi</i>			
	<i>TpN47 (224-351)</i>				<i>TpN47 (352-434)</i>			
	70ng/ml				70ng/ml			
	72h 4°C	72h 42°C	signal recovery		72h 4°C	72h 42°C	signal recovery	
R2	<i>EcSlyD-EcSlyD-TpN47 [C] -Ru</i>				<i>EcSlyD-EcSlyD-TpN47 [D] -Ru</i>			
	<i>TpN47 (224-351)</i>				<i>TpN47 (352-434)</i>			
	70ng/ml				70ng/ml			
	72h 4°C	72h 42°C	signal recovery		72h 4°C	72h 42°C	signal recovery	
Thermal stress	cts	SD	cts	SD	cts	SD	cts	SD
	619	1.01	658	1.03	1,417	3.02	1,424	3.03
	618	1.01	665	1.04	1,684	3.59	1,673	3.56
	643	1.06	660	1.03	5,031	10.7	5,133	10.9
	612	1.00	670	1.05	4,009	8.54	4,180	8.91
	655	1.07	669	1.05	2,264	4.82	2,370	5.05
	624	1.02	659	1.03	2,301	4.90	2,366	5.04
	623	1.02	655	1.03	2,733	5.82	2,844	6.06
	607	1.00	648	1.01	460	0.98	459	0.98
	614	1.01	626	0.981	475	1.01	480	1.02
	610	1.00	645	1.01	469	1.00	468	1.00
	608	1.00	635	0.995	472	1.01	471	1.00
	610		638		469		469	
average negative	610		638		469		469	

Figure 11/12

Figure 12/12

Table 6

R1	EcSlyD-EcSlyD-TpN47[CD] -Bi					EcSlyD-EcSlyD-TpN47[ABCD] -Bi				
	TpN47 (224-434)					full-length TpN47 (21-434)				
	70ng/ml					70ng/ml				
	72h 4°C	72h 42°C	signal recovery			72h 4°C	72h 42°C	signal recovery		
R2	EcSlyD-EcSlyD-TpN47[CD] -Ru					EcSlyD-EcSlyD-TpN47[ABCD] -Ru				
	TpN47 (224-434)					full-length TpN47 (21-434)				
	70ng/ml					70ng/ml				
	72h 4°C		72h 42°C		signal recovery	72h 4°C		72h 42°C		signal recovery
thermal stress	cts	SD	cts	SD		cts	SD	cts	SD	
BM 200830	1,803	3.41	1,442	2.67	80.0%	4,250	8.50	2,217	4.08	52.2%
BM 200831	2,272	4.30	1,775	3.29	78.1%	5,383	10.8	2,609	4.80	48.5%
BM 202017	7,532	14.3	5,116	9.48	67.9%	77,703	155	44,871	82.5	57.7%
BM 202018	5,967	11.3	4,044	7.49	67.8%	62,711	125	36,044	66.3	57.5%
BM 206252	3,531	6.68	2,550	4.72	72.2%	22,806	45.6	10,179	18.7	44.6%
BM 206253	3,465	6.56	2,565	4.75	74.0%	23,101	46.2	10,556	19.4	45.7%
BM 206254	4,231	8.01	2,958	5.48	69.9%	29,093	58.2	13,151	24.2	45.2%
Trina #097	531	1.006	536	0.993	101%	492	0.984	540	0.992	110%
Trina #098	531	1.005	539	0.998	101%	499	0.998	546	1.00	109%
Trina #099	526	0.995	548	1.014	104%	505	1.011	547	1.01	108%
Trina #100	525	0.994	537	0.995	102%	503	1.006	542	0.997	108%
average negative	528		540			500		544		

Figure 12/12