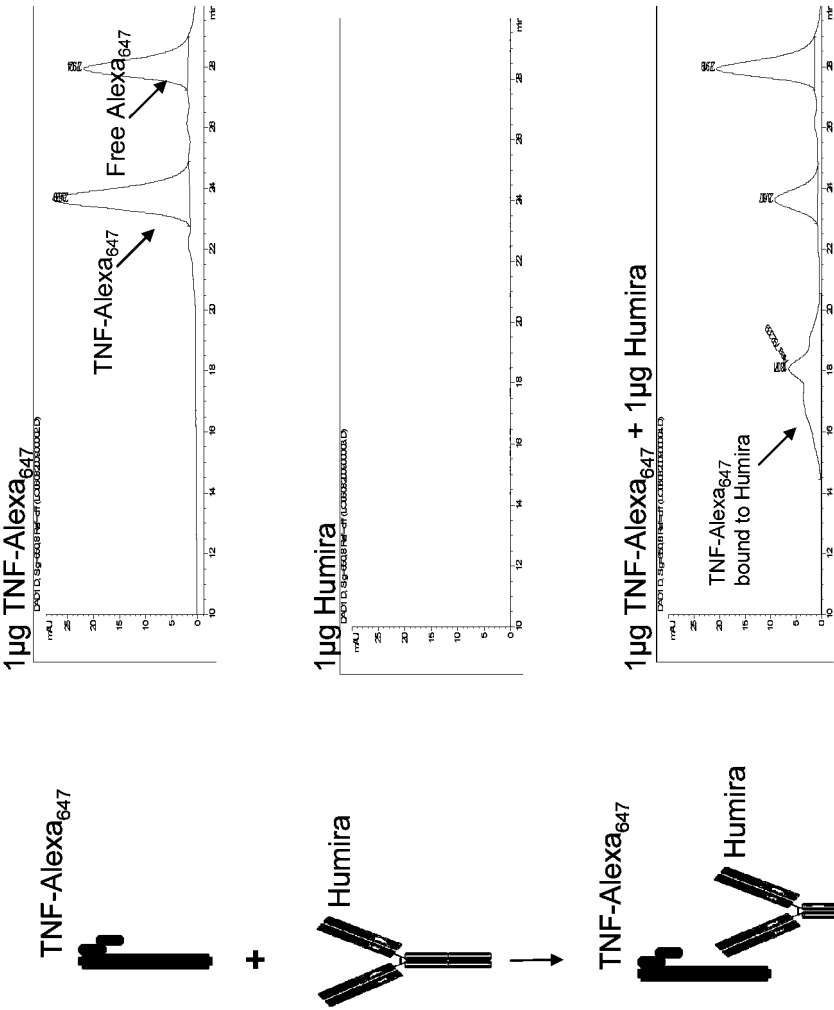




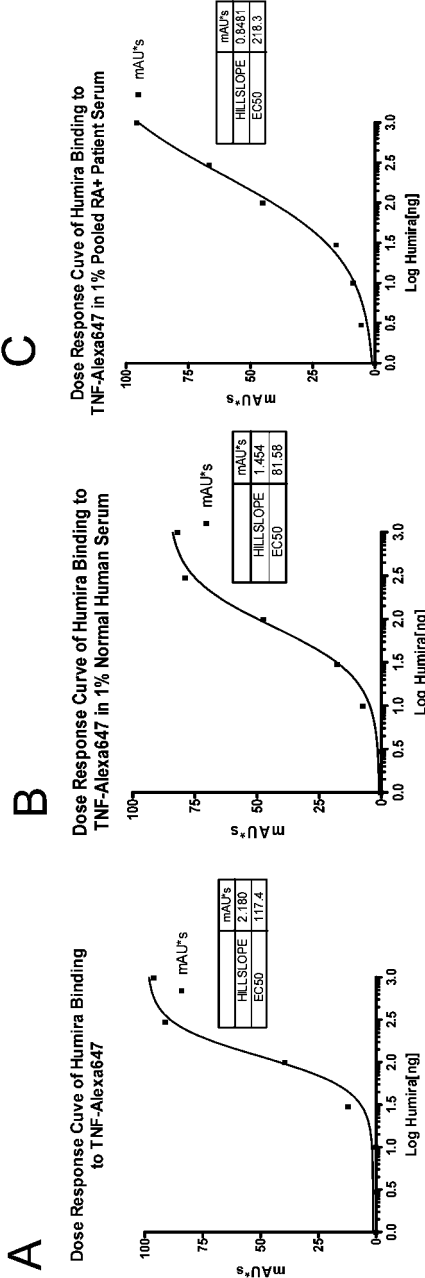


FIG. 2

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Bridging Assay:

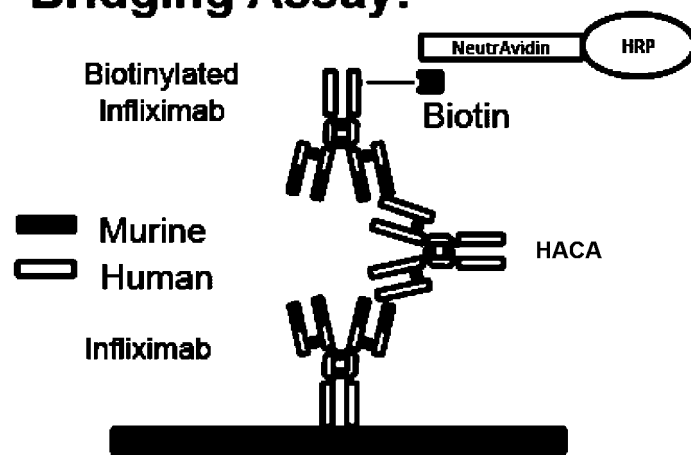


FIG. 3

- (1) Preparation of Alexa⁶⁴⁷-labeled Remicade
- (2) Incubation with patient serum (HACA) to form the complex
- (3) Quantification of the complex by SE-HPLC
- (4) Assay works in the presence of remicade

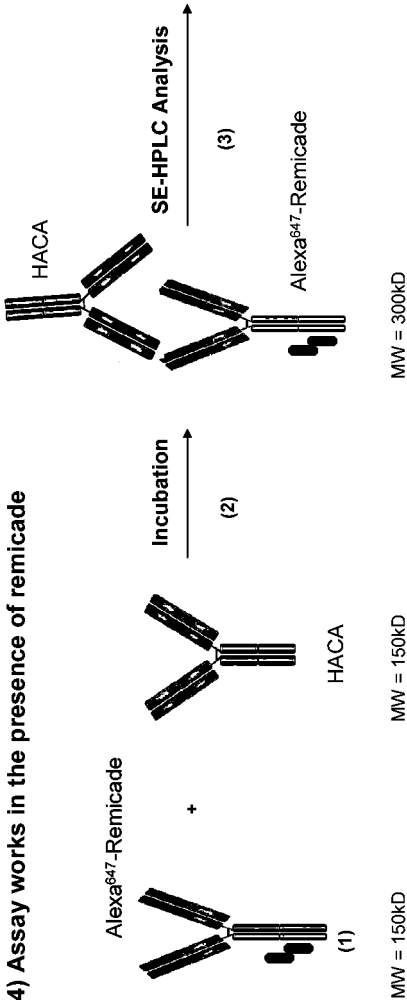


FIG. 4

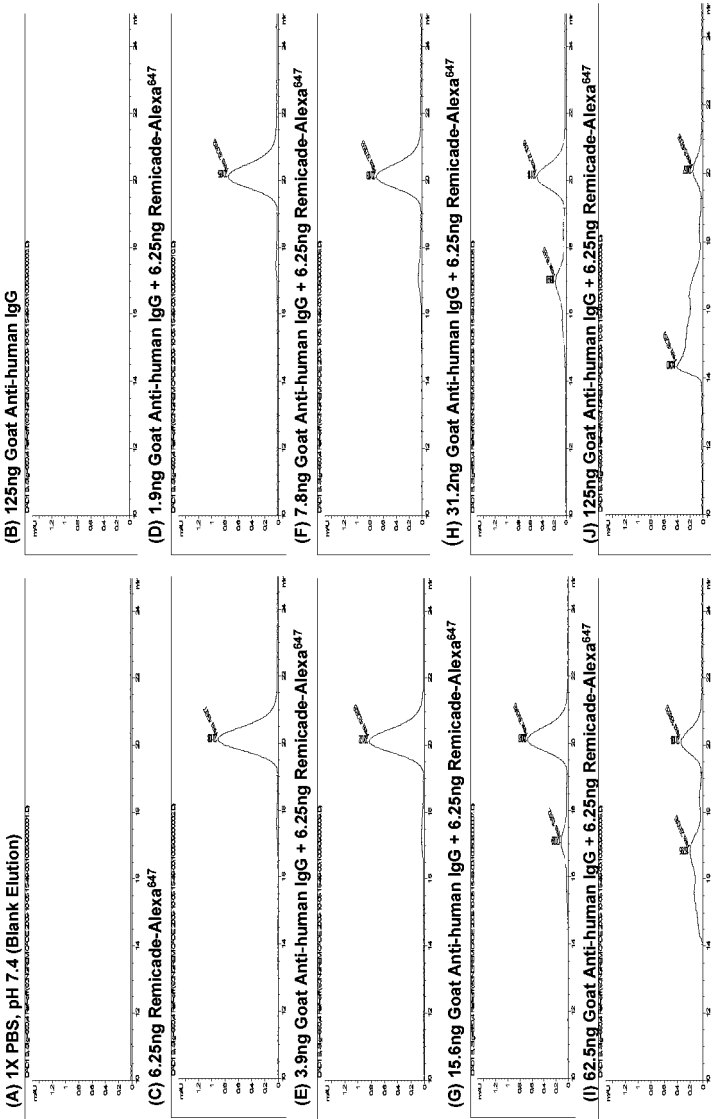


FIG. 5

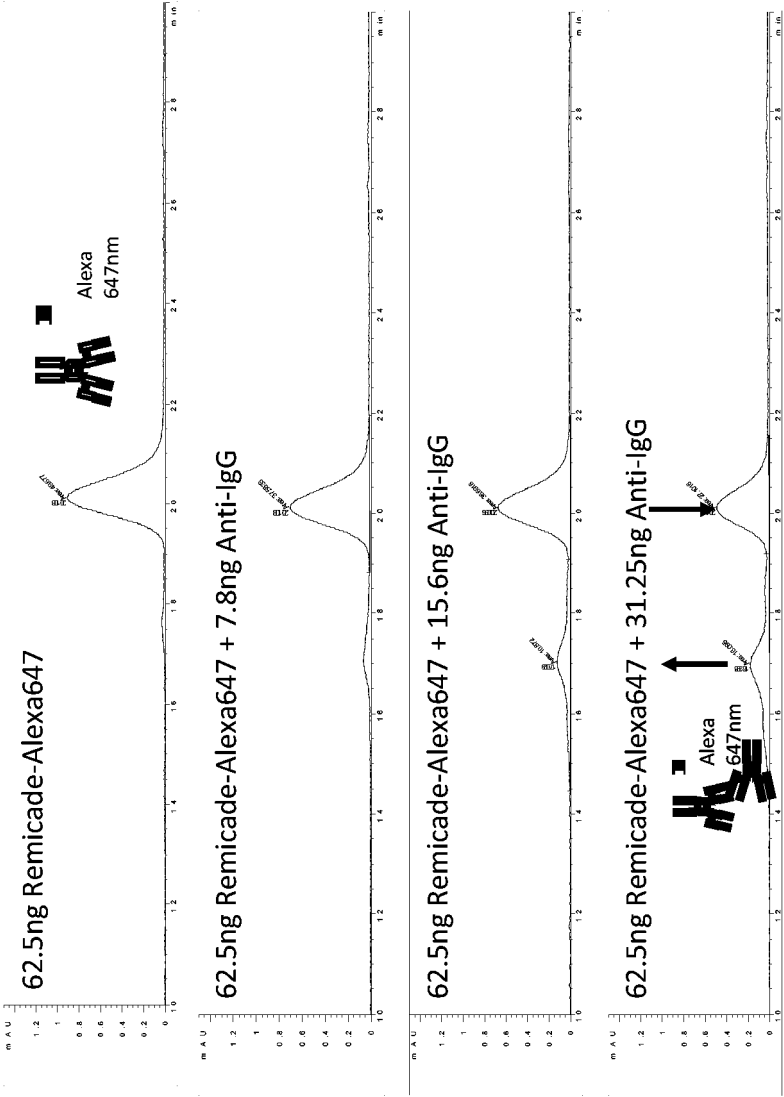


FIG. 6

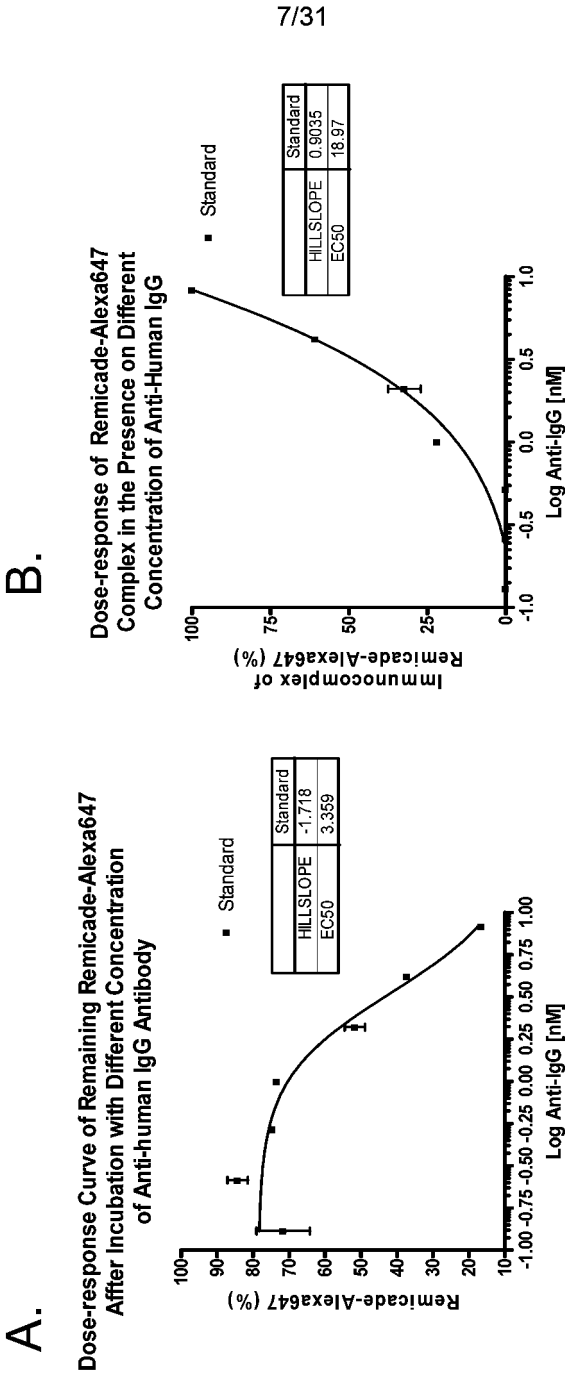
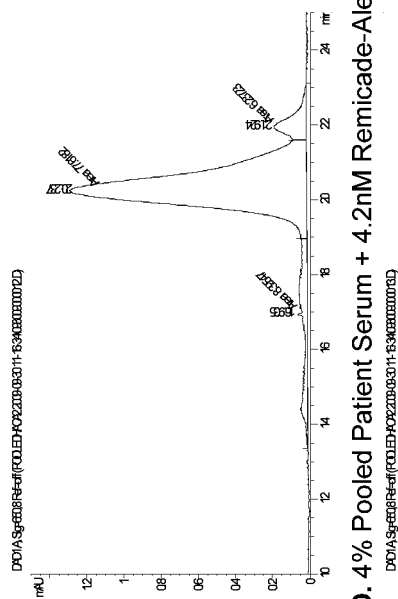
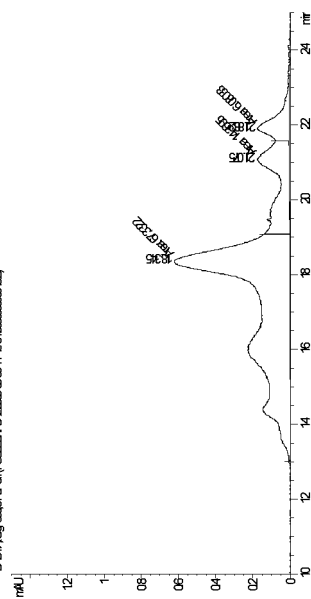


FIG. 7

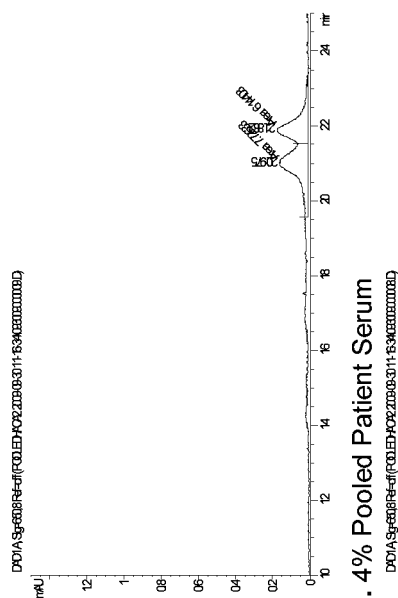
B. 4% Normal Human Serum + 4.2nM Remicade-Alexa



D. 4% Pooled Patient Serum + 4.2nM Remicade-Alexa



A. 4% Normal Human Serum



C. 4% Pooled Patient Serum

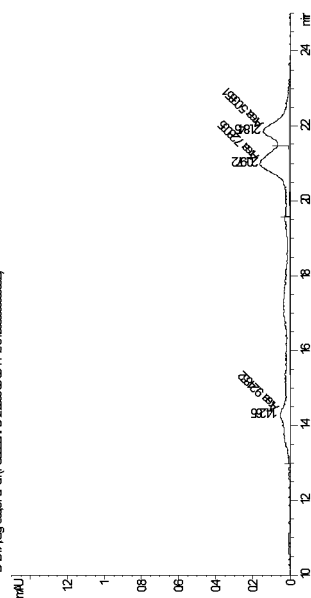


FIG. 8

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Accession #	Bridge ELISA		Mobility Shift Assay (4% Serum)		
	Quantitative Result	Qualitative Result	HACA Shift Assay	HACA Area/Remicade-647 Area	Remicade (nM)
SK07010477	22.26	Positive	Positive	0.78	3.33
SK07060083	1.41	Negative	Negative	0.1	4.06
SK07070083	1.41	Negative	Negative	0.1	8.81
SK07070305	1.41	Negative	Positive	0.46	7.34
SK07070595	1.41	Negative	Positive	0.25	8.35
SK07071213	2.48	Positive	Positive	0.16	5.30
SK07081127	22.07	Positive	Positive	0.28	3.00
SK07110035	1.41	Negative	Positive	0.18	>66.7
SK07141447	2.62	Positive	Positive	0.42	2.43
SK07171059	10.11	Positive	Positive	18.02	2.59
SK07171095	10.03	Positive	Positive	0.24	2.24
SK07210210	9.26	Positive	Positive	0.8	<0.67
SK07231216	25.58	Positive	Positive	Complete Shift	1.34
SK07310149	2.74	Positive	Positive	0.21	<0.67
SK08040168	22.21	Positive	Positive	Complete Shift	<0.67
SK08051035	9.72	Positive	Positive	8.7	1.89
SK08070307	2.49	Positive	Positive	0.23	3.14
SK08120222	9.2	Positive	Positive	0.25	<0.67
SK08260093	23.15	Positive	Positive	0.48	1.04
SK08260783	2.67	Positive	Positive	0.25	3.30
62.5ng Remicade-647				0.12	
100ng TNF-647					<0.67

FIG. 9

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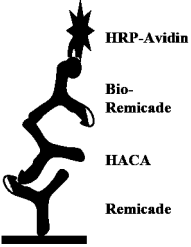
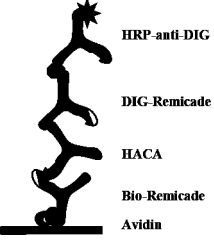
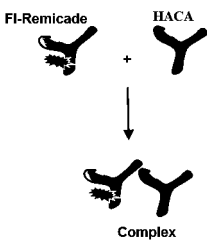
	Bridge HACA Assay	Biotin and DIG-Based Homogeneous Bridging ELISA	HACA Assay of the Present Invention
Assay Format	 HRP-Avidin Bio-Remicade HACA Remicade	 HRP-anti-DIG DIG-Remicade HACA Bio-Remicade Avidin	 FI-Remicade + HACA ↓ Complex
Non-specific Background Interference	High	High	Low
Sensitivity	Low	Medium	High
Possibility of False-Positive and False-Negative	High	High	Low
IgG4 HACA Detection	No	No	Yes
Ig Isotype identification	No	No	Yes
Tolerance of Drug in the Sample	Poor	Poor	Good

FIG. 10

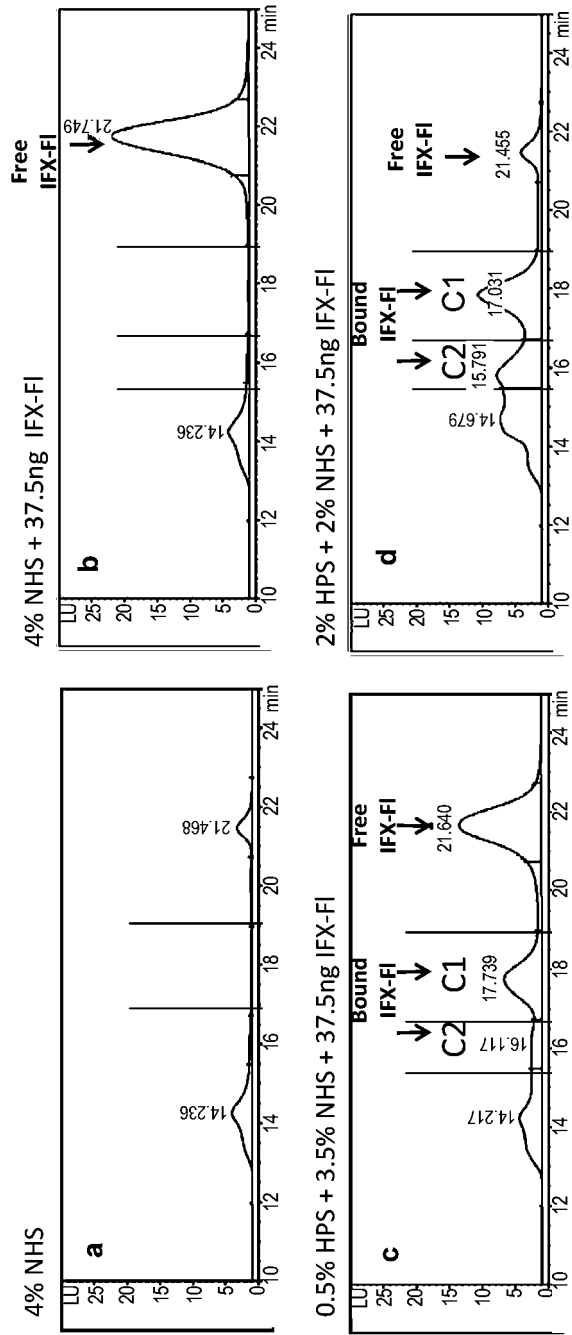


FIG. 11

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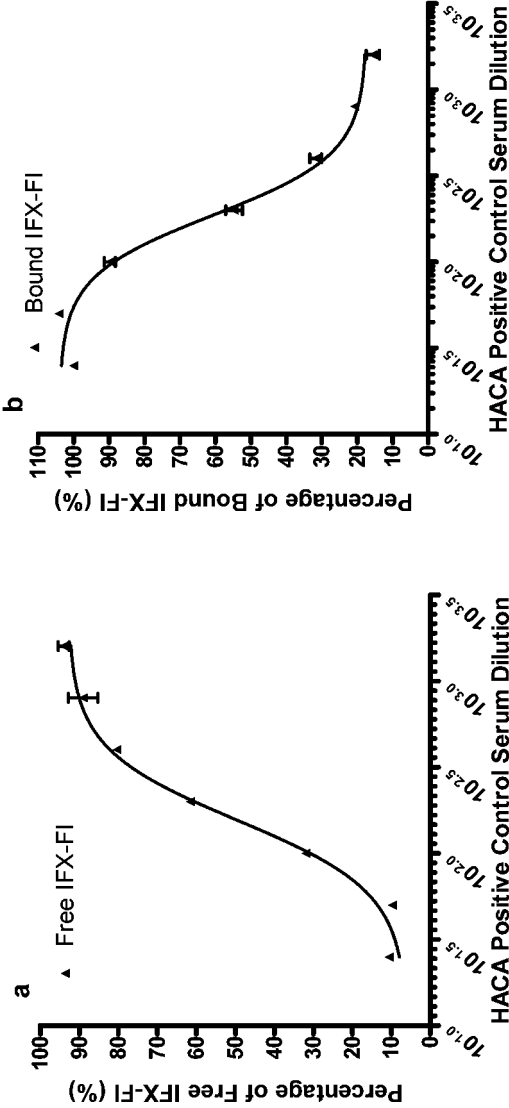


FIG. 12

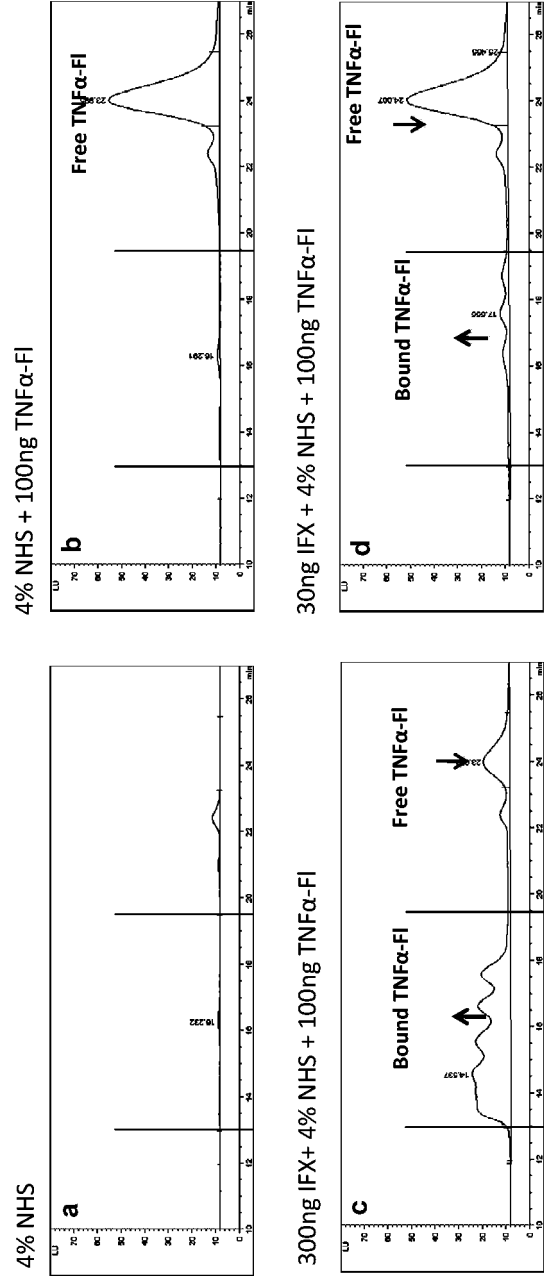


FIG. 13

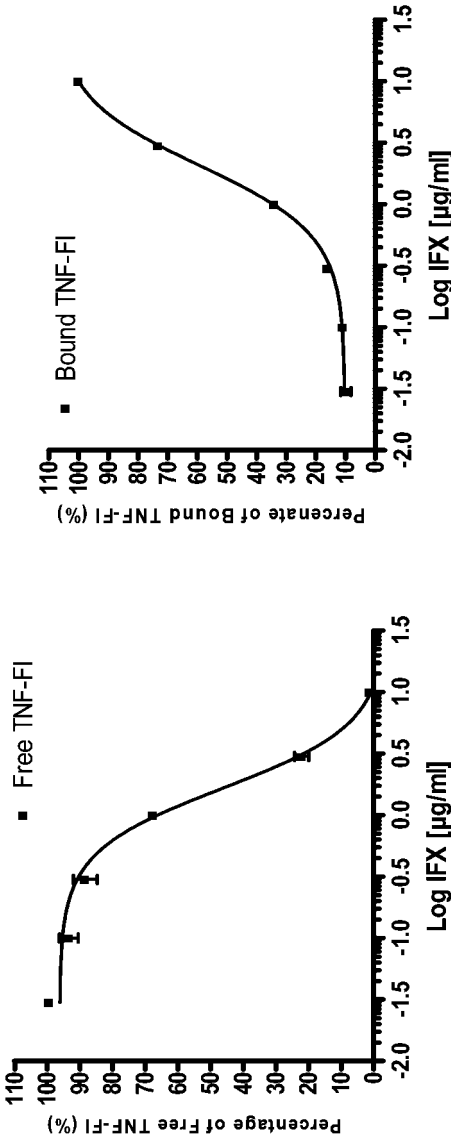


FIG. 14

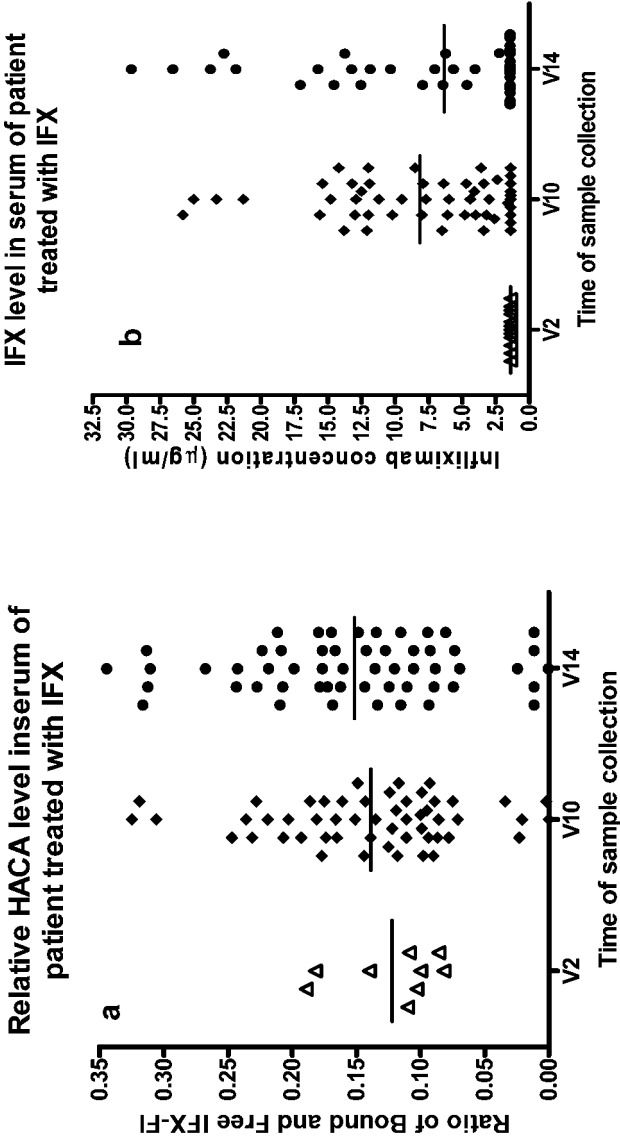


FIG. 15

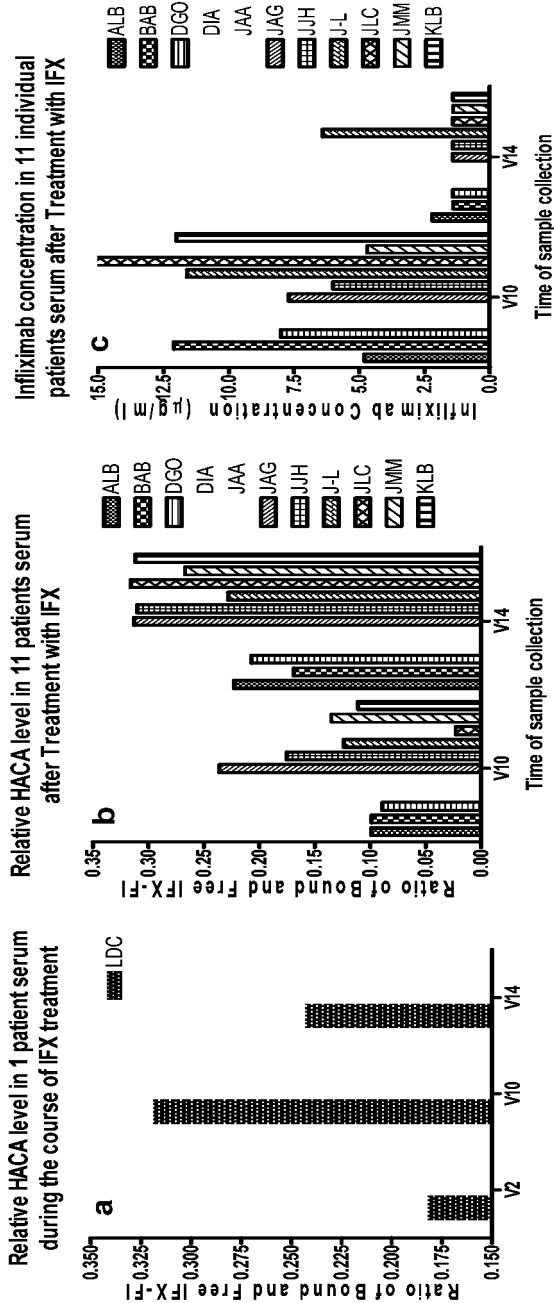
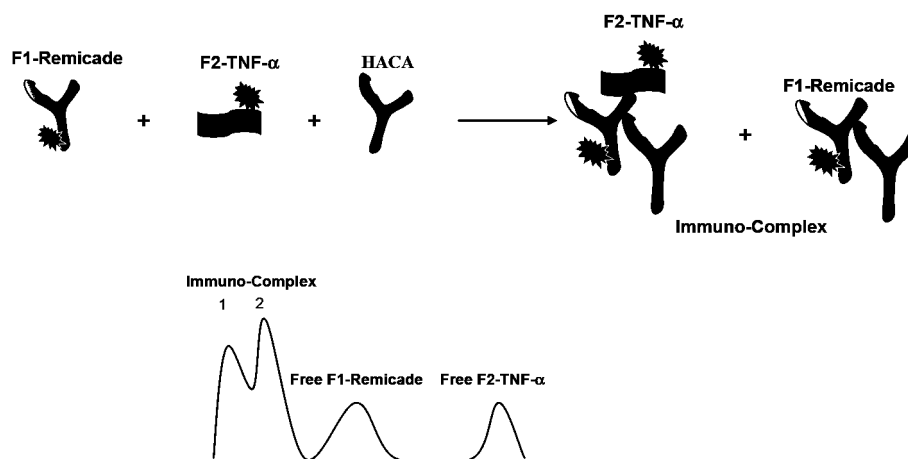
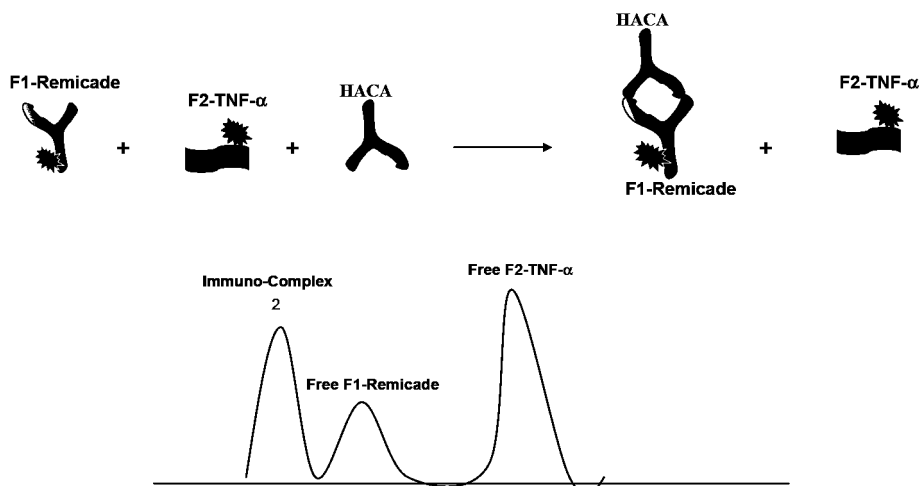


FIG. 16

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A. Non-neutralizing HACA Assay**B. Neutralizing HACA Assay****FIG. 17**

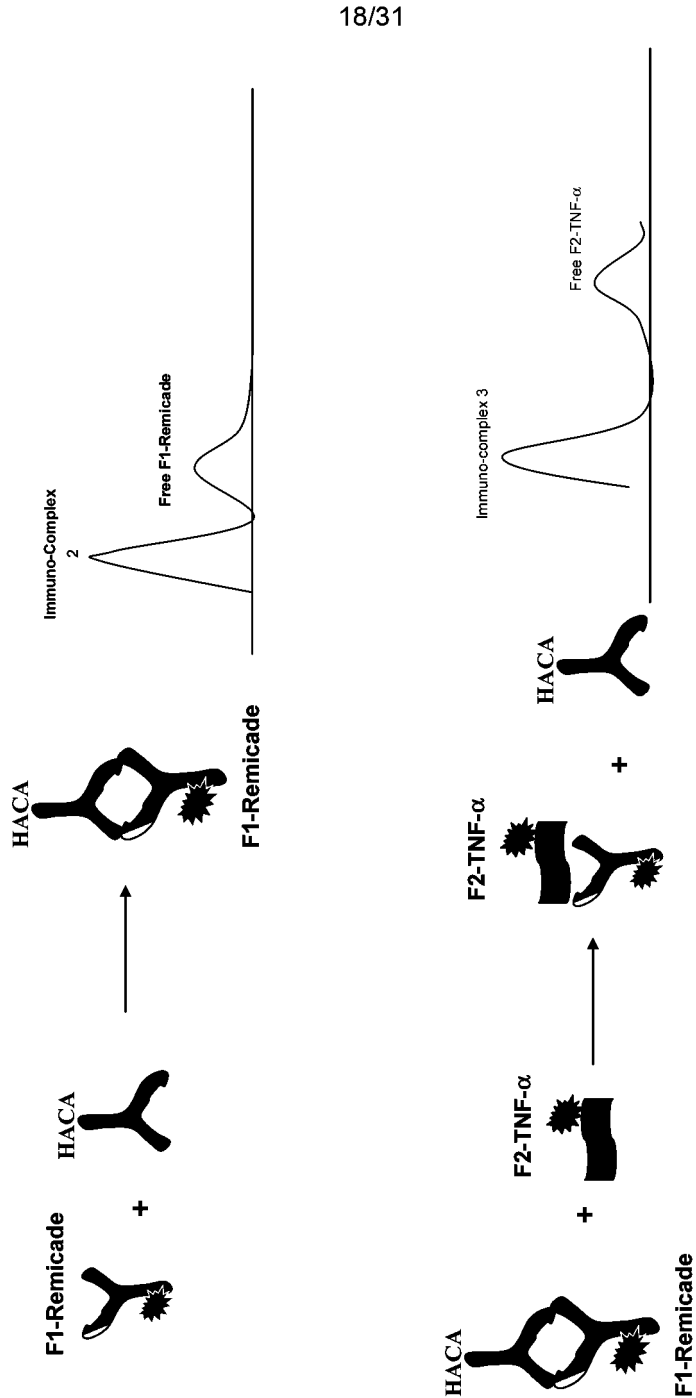


FIG. 18

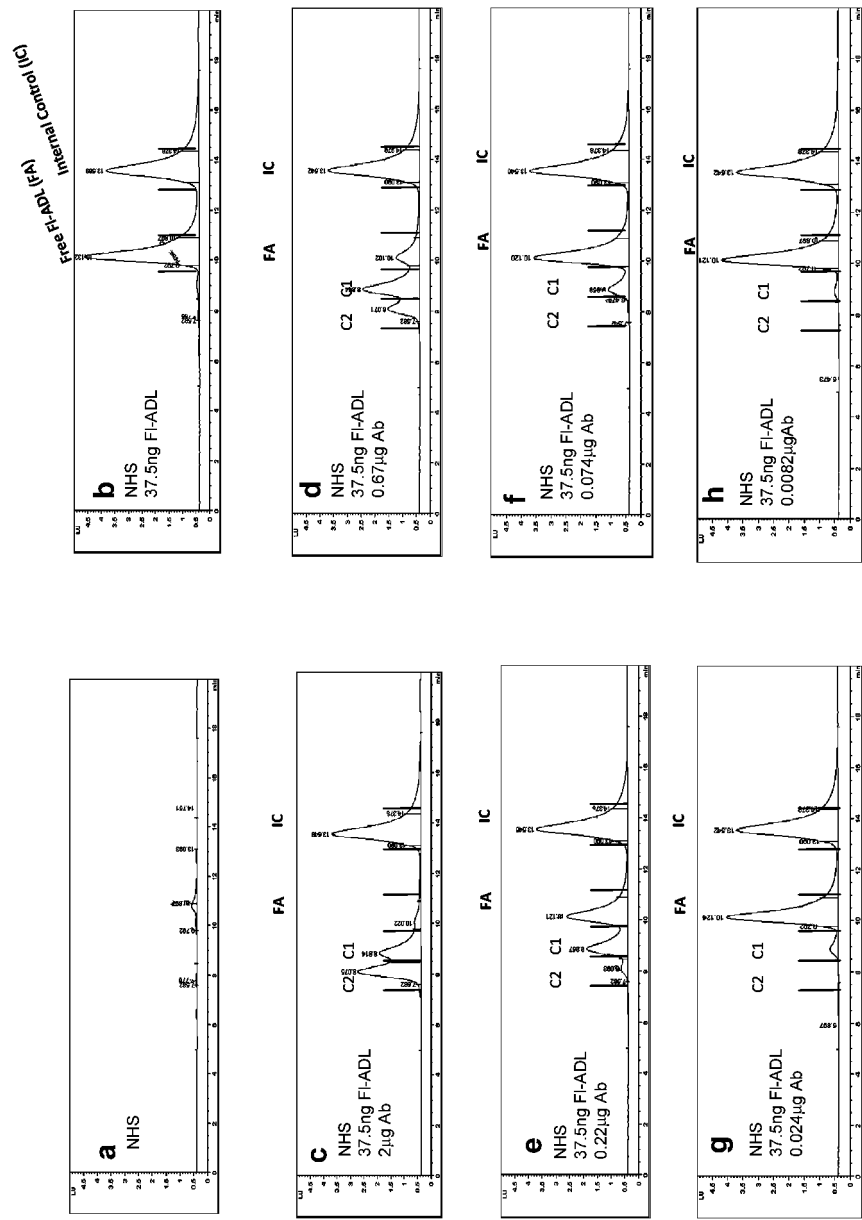


FIG. 19

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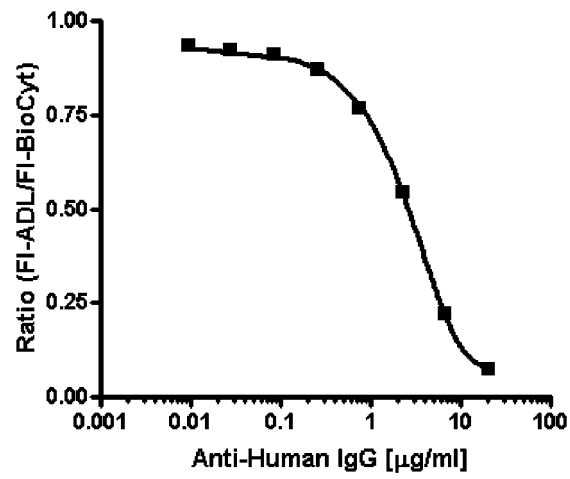


FIG. 20

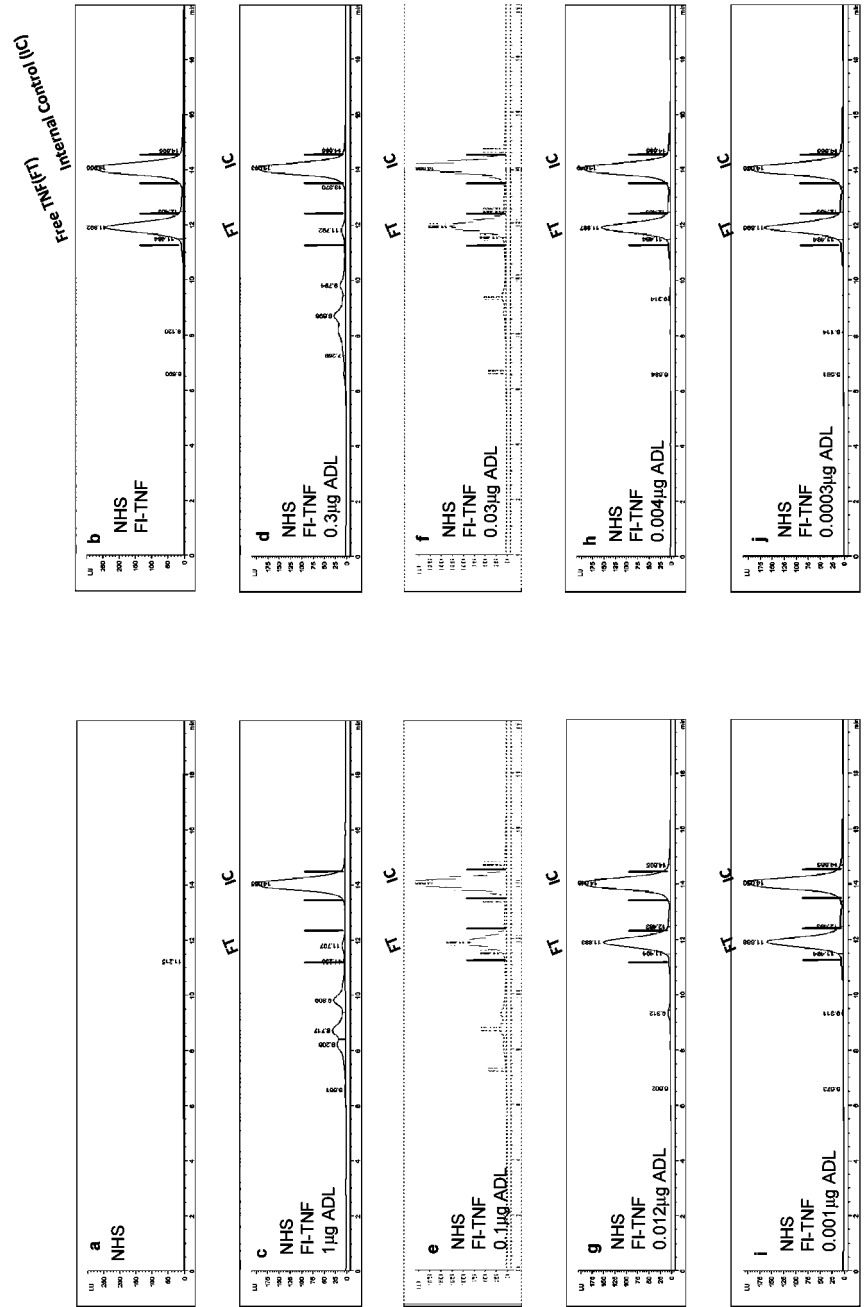
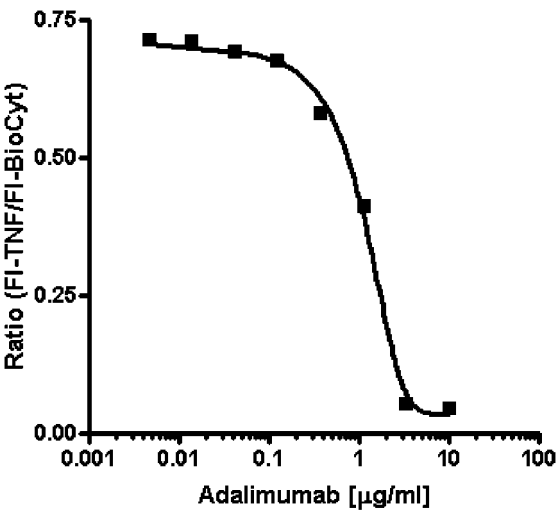


FIG. 21

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HILLSLOPE	-1.813
EC50	1.213

FIG. 22

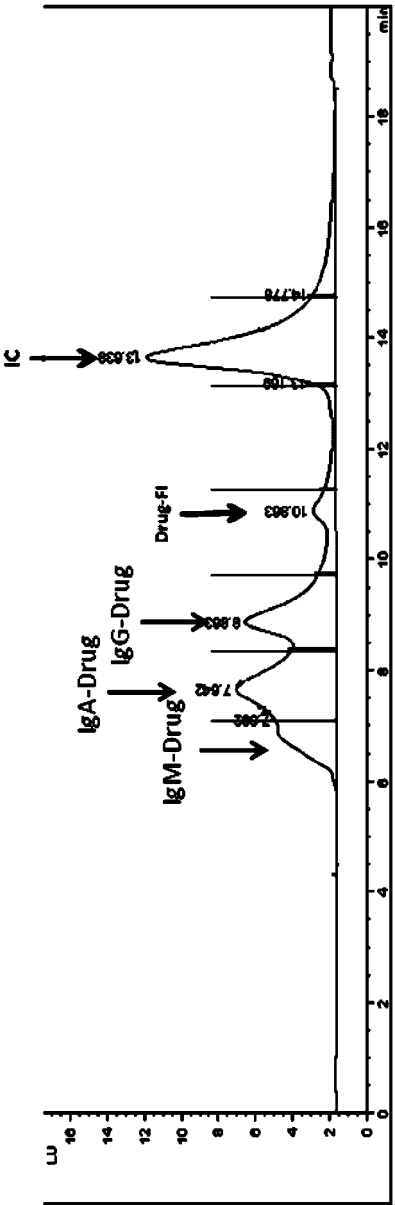
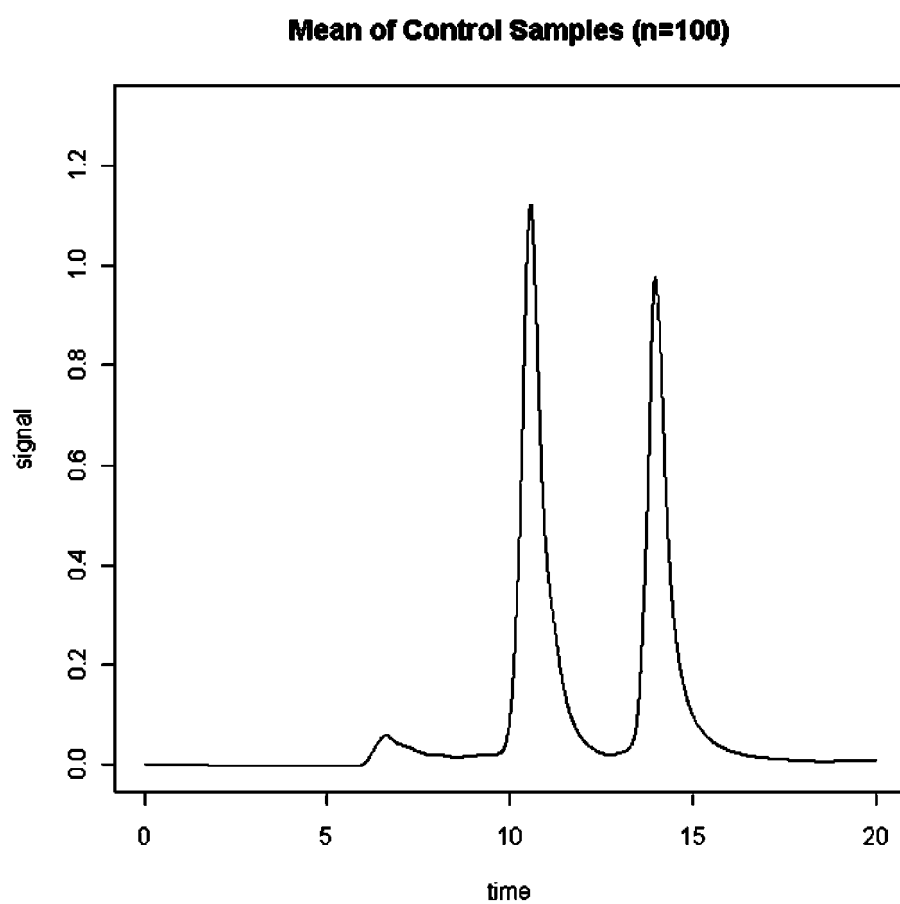
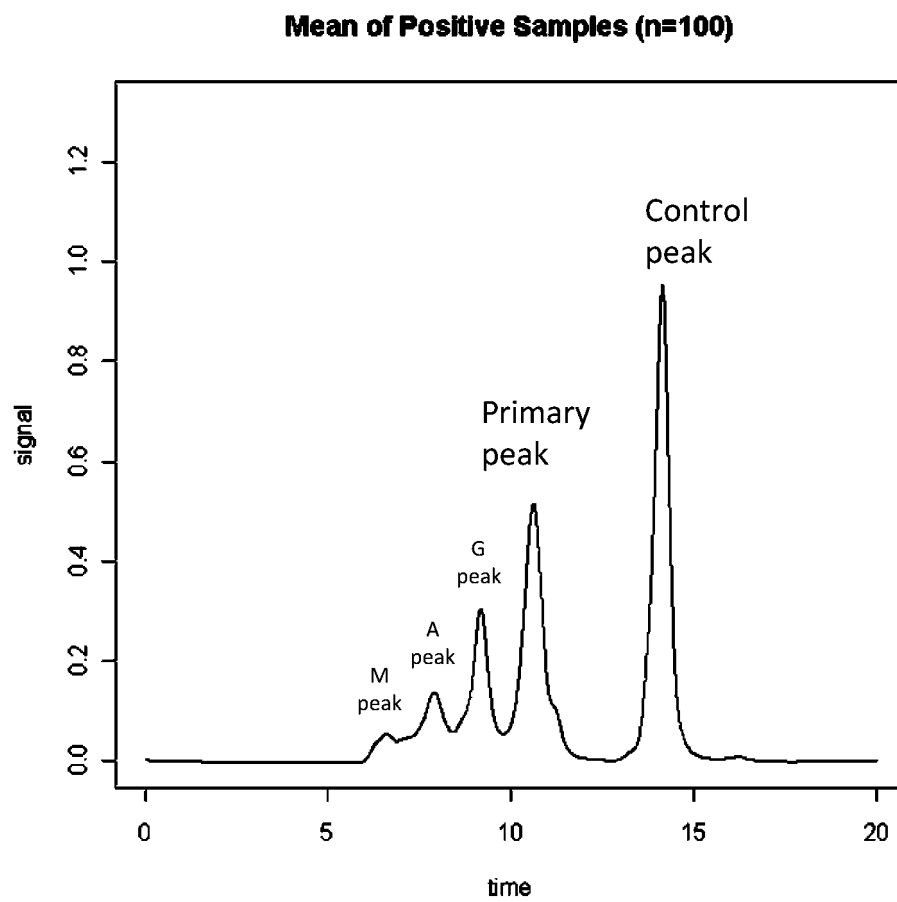


FIG. 23

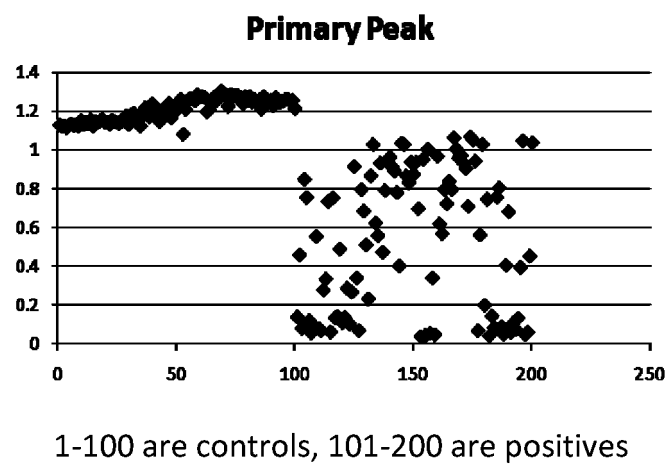
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**FIG. 24**

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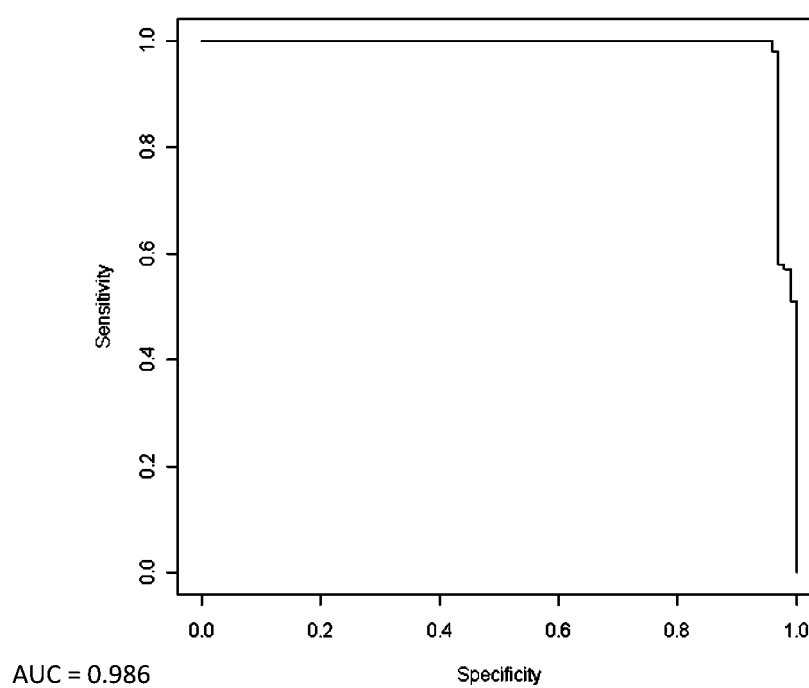
**FIG. 25**

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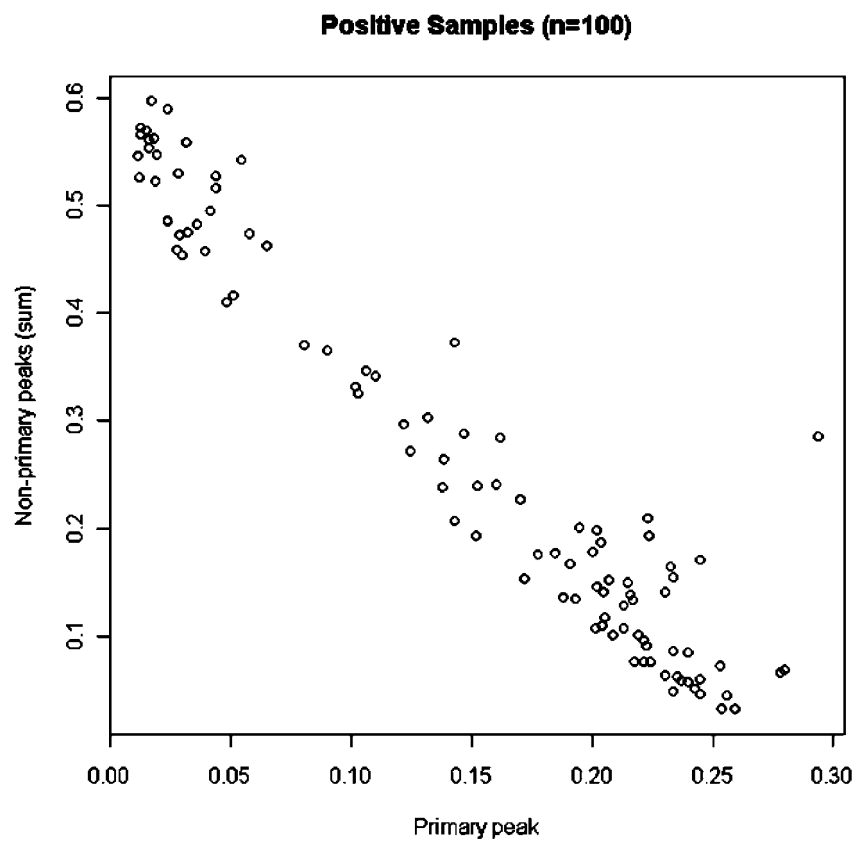
**FIG. 26**

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ROC Curve for primary peak

**FIG. 27**

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**FIG. 28**

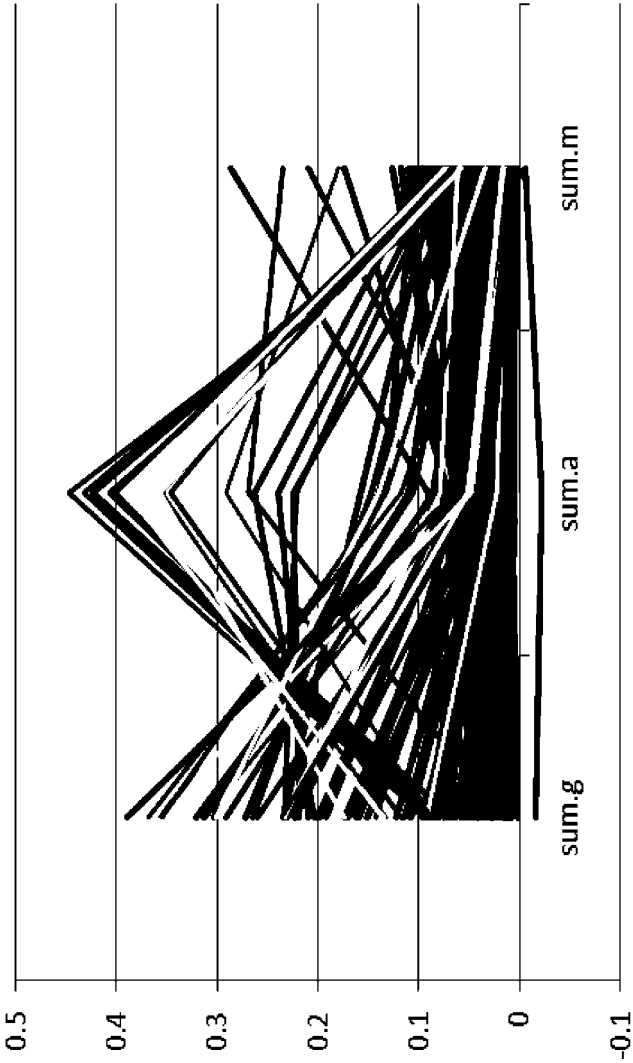
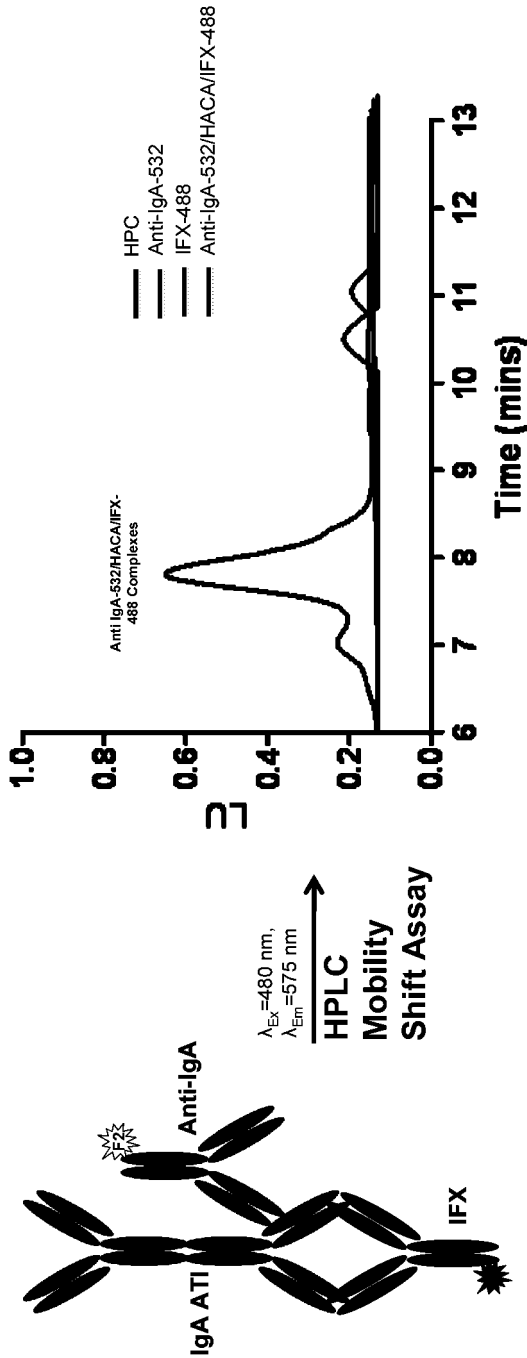


FIG. 29



- Alexa532 is excited by Alexa488 only when fluorophores are in close proximity
- (Anti-Ig/HACA/IFX complex)
- Isotypes determined by using different Anti-Igs labeled with Alexa532

FIG. 30

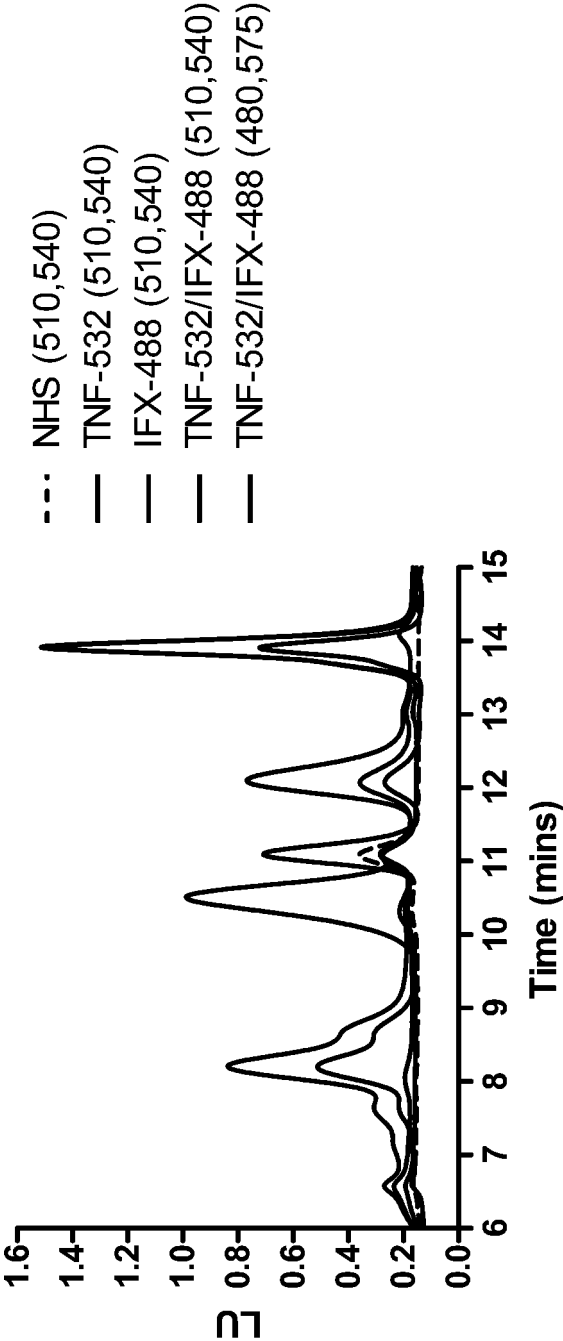


FIG. 31