From Analysis of Antibodies Selection to Ultra Sensitivity Biomarker Detection [Case Study: Neurofilament light (NFL)]

In the field of neurological diseases, the identification and quantification of axonal damage from peripheral blood have become crucial for diagnostic and prognostic assessments of various neuropsychiatric conditions. Neurofilament light chain (NFL) has emerged as a stable biomarker released from neuro-axonal damage in different neurological disorders. However, current methods for analyzing blood NFL levels rely on less specific enzyme-linked immunosorbent assay (ELISA) techniques or more expensive single-molecule array (SIMOA) methods.

In this study, a novel approach called fiber optic nanogold-linked immunosorbent assay (FONLISA) was developed using the fiber optic particle plasmon resonance (FOPPR™) technique. This innovative method aims to accurately measure the concentrations of peripheral blood NFL. By employing FONLISA in conjunction with FOPPR™, it is anticipated that a more specific and cost-effective alternative can be provided for assessing NFL levels in neurological disorders.

Table 1. Antibody from different brands

Antibody Company	Clonal
Abcam (1)	Monoclonal
Abcam (2)	Monoclonal
GeneTex (1)	Monoclonal
GeneTex (2)	Monoclonal
GeneTex (3)	Polyclonal
IReal (1)	Polyclonal
IReal (2)	Polyclonal
Uman (1)	Monoclonal
Uman (2)	Monoclonal

Antigen Company	Sequence
Abcam	Full Length

Table 2. The affinity constant from different brands

Antigen	Antibody								
abcam	Abcam (1)	Abcam (2)	GeneTex (1)	GeneTex (2)	GeneTex (3)	IReal (1)	IReal (2)	Uman (1)	Uman (2)
k_a (1/MS)	1.35×10^{5}	3.01×10^{5}	2.57×10^{4}	7.98×10^{4}	1.68×10^{5}	5.54×10^{4}	2.20×10^{5}	2.42×10^{5}	5.28×10^{4}
k_d (1/s)	2.64×10^{-4}	1.60×10^{-3}	5.20×10^{-4}	2.93×10^{-3}	1.54×10^{-3}	2.06×10^{-3}	3.18×10^{-4}	6.01×10^{-4}	7.70×10^{-4}
K_D (nM)	1.96	5.34	20.26	36.79	9.19	37.24	1.44	2.48	14.58

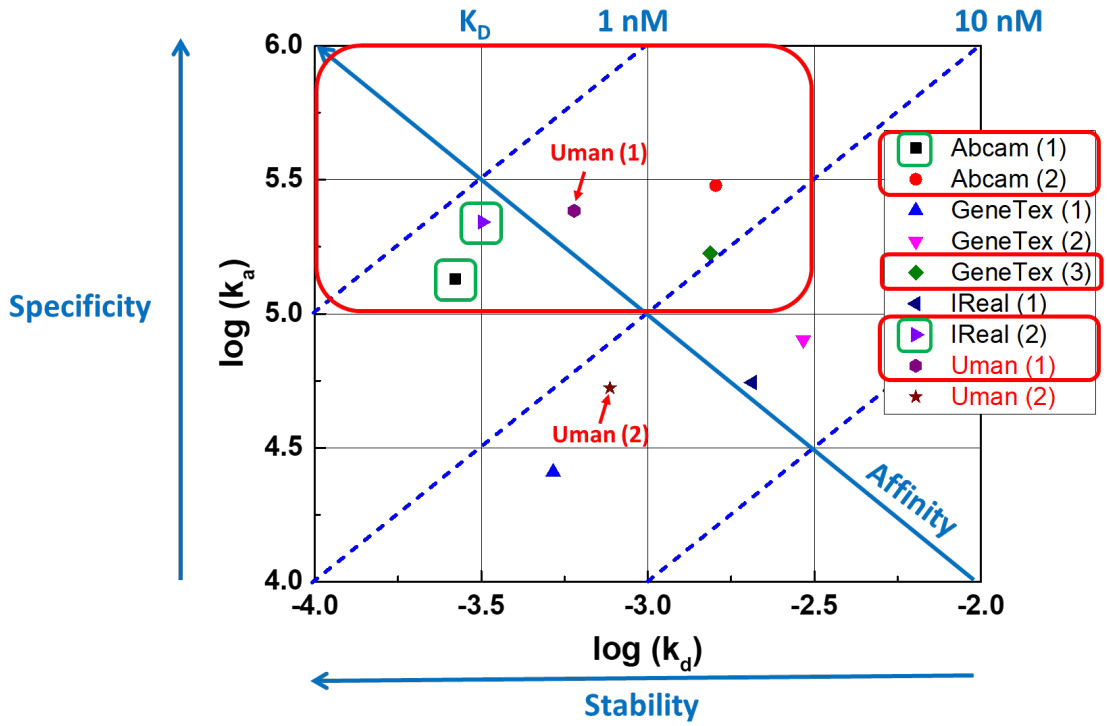


Fig 1. The affinity constant distribution of nine antibody

Based on the affinity analysis results of nine antibody brands, the red square indicates that five antibody brands exhibited better affinity constants, with values smaller than 10 nM. Among these five brands, Abcam (1) and IReal (2) demonstrated better stability. However, since Uman (1) and Uman (2) are antibodies with application patents owned by another company, the selection of the remaining antibody will be determined after conducting the epitope binning test.

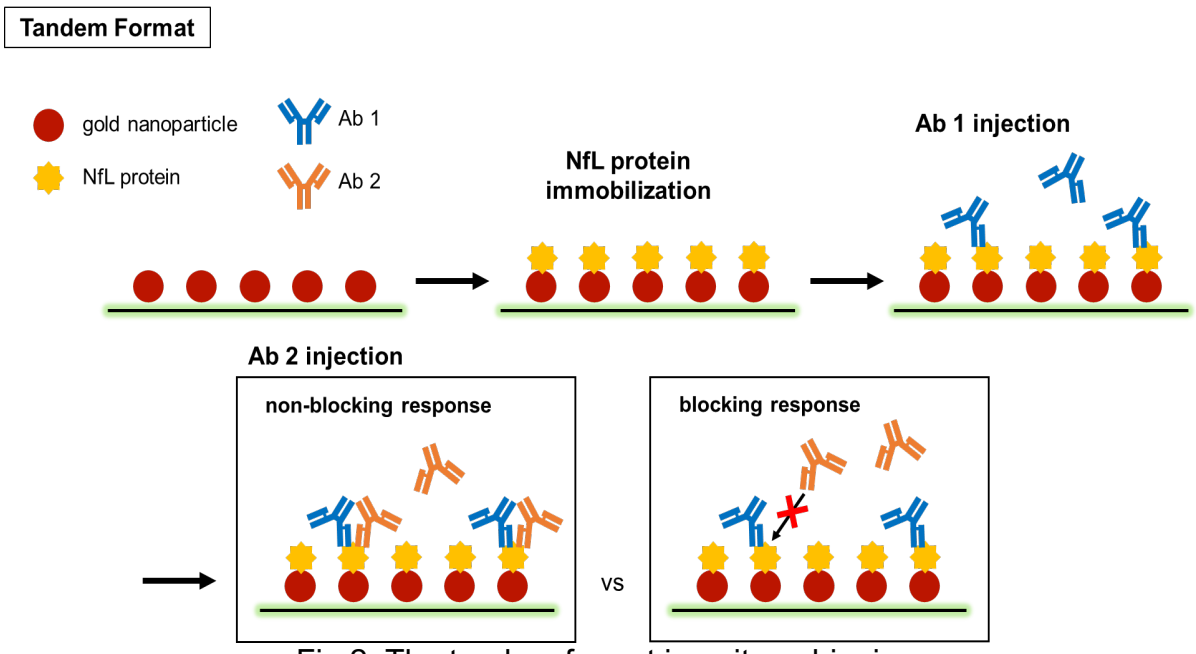


Fig 2. The tandem format in epitope binning

In the tandem format of the epitope binning test, the Nfl protein is initially immobilized on the fiber. Subsequently, the first antibody is injected, followed by a wash step. Finally, the second antibody is injected. If the second antibody is blocked by the first antibody, there will be no change in the signal observed in the sensorgram. However, if the two antibodies do not block each other, there will be a noticeable change in the signal.

(a) (b)

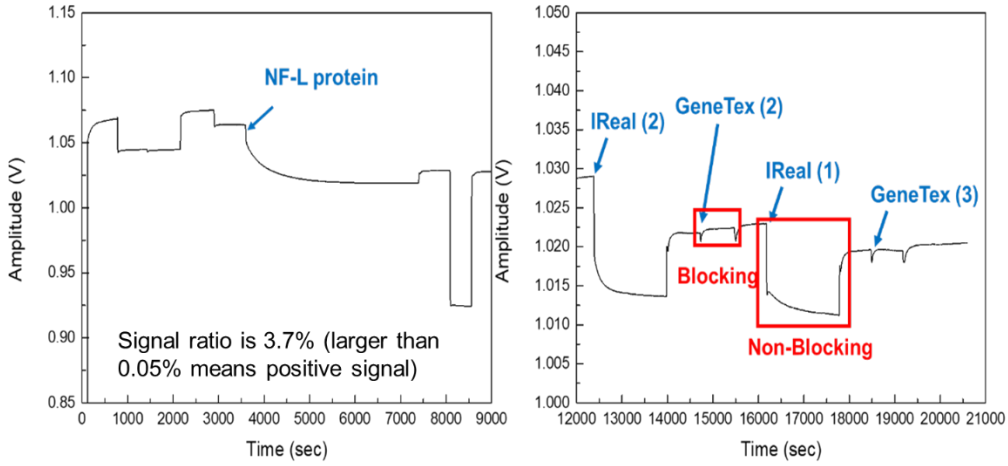


Fig 3. The results of the epitope binning test. (a) the Nfl protein is immobilized on the fiber (2) four different antibodies are tested on the same chip. It is observed that GeneTex (2) shares an epitope with one of the IReal (2) antibodies. On the other hand, IReal (1) antibodies have epitopes that are distinct from both IReal (2) and GeneTex (2). Additionally, GeneTex (3) shares the same epitope as GeneTex (2) and one of the IReal (1) antibodies.

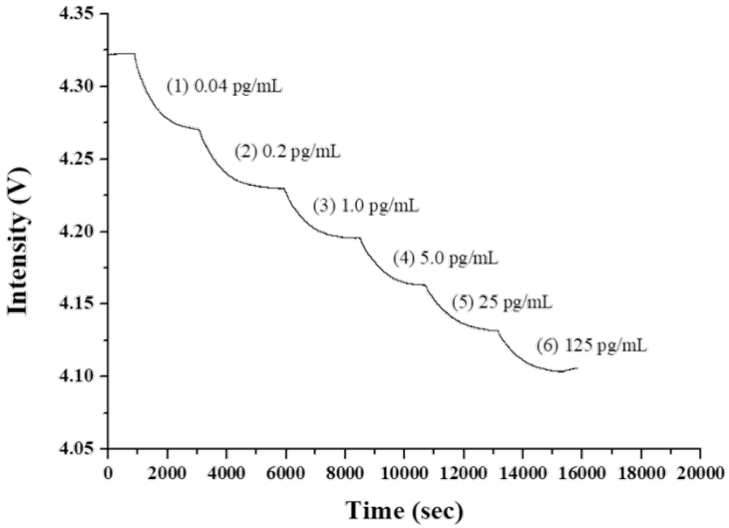
Nfl Antibody		Second Antibody						
		Abcam (1)	Abcam (2)	GeneTex(1)	GeneTex(2)	GeneTex(3)	IReal (1)	IReal (2)
First Antibody	Abcam (1)							
	Abcam (2)							
	GeneTex(1)							
	GeneTex(2)							
	GeneTex(3)							
	IReal (1)							
	IReal (2)							

blocking
partial blocking
non-blocking

Fig 4. The Epitope Map of seven antibodies. By integrating the results of the affinity analysis and epitope binning, it is determined that Abcam (1) and IReal (2) antibodies are the suitable antibody pairs for the assay development. Although **Abcam (1)** antibody has the second-best affinity constant, it demonstrates **superior stability**. Moreover, it is a **monoclonal** antibody. On the other hand, **IReal (2)** antibody exhibits the **best affinity constant**, despite being a **polyclonal** antibody. Given these findings, Abcam (1) can serve as the capture antibody, while IReal (2) antibody can function as the detection antibody.

After selecting the appropriate antibody pairs, they were utilized for further assay development. Firstly, the standard curve was generated with duplicated test results showed in Figure 5. The standard curve demonstrates a linear range of 0.04 pg/mL to 125 pg/mL, covering a dynamic range of four orders of magnitude. The limit of detection (LOD) for the Nfl assay was determined to be **3.4 fg/mL.**

(a)



(b)

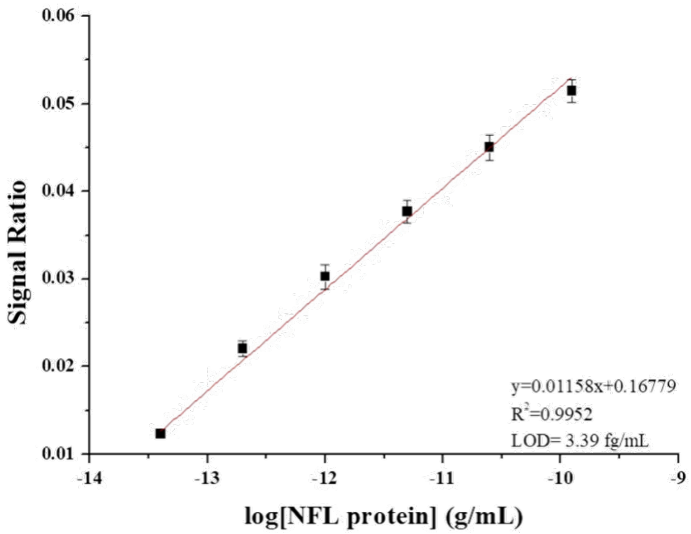


Fig 5. (a) Real-time detection upon injection of different standard NFL concentrations ranging from **0.04 pg/mL to 125 pg/mL (~4 orders)**. (b) Calibration curve for standard NFL using FONLISA method of FOPPR biosensor (n = 2). **(LOD = 3.39 fg/mL)**