



APTAMER INFORMATION

Mouse CD8 aptamer H.11 #538

1a. Description:

- **Identifiers:** H.11 (oligo # 538)
- **Number of DNA nucleotides:** 32 bases (without 3'-6T), 38 bases (with 3'-6T)
- **Molecular weight (including 3'-6T and biotin):** 12,121 g/mol
- **Target for selection:** Recombinant Mouse CD8A protein, Sino Biological Inc. (Cat #50389-M08H)

Aptamer was selected from a randomized 32-mer library against Mouse CD8A protein. Proprietary methods were then used to select the aptamer.

Aptamer folding instruction before use:

Once the aptamer is in its working concentration, it needs to be heated to 85-90 °C for 2 minutes, and then cooled to room temperature before use.

1b. Validation data with Mouse CD8 protein by BLI (Bio-Layer Interferometry) method:

- **Immobilized Ligand:** Mouse CD8 aptamer #538 with 3'-6T and biotin
- **Analyte:** Mouse CD8 protein
- **Buffer used for validation:** 1X PBS pH 7.4
- **Random aptamer as negative control:** Random aptamer of same length, similar molecular weight and same 3'-modifications (i.e 6T and biotin) as Mouse CD8 aptamer.

1c. Kinetics Screening Assay using Streptavidin Biosensors :

By two types of reference methods, we validate the binding data.

- **Single reference data:** All curves are referenced to a sensor dipped in buffer alone (no protein) (see Figures 1 and Table 1).
- **Double reference data:** For even more stringent validation of aptamer binding, we “double-reference” all data to both protein-free buffer (as above) as well as to a sensor with a control or “sham” aptamer (see Figures 1, 3 and Table 2). This random aptamer controls for any non-specific charge-only interactions.



1d. Single reference data:

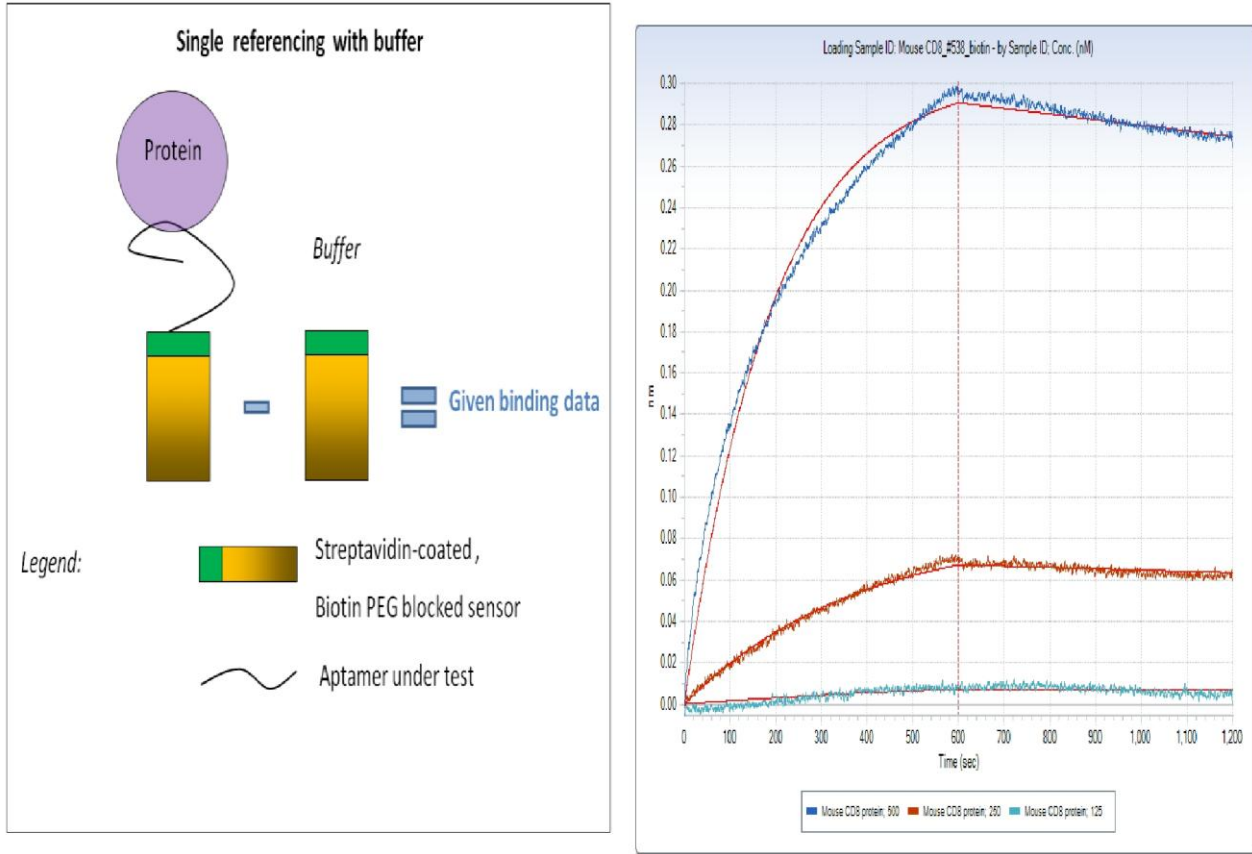


Figure 1. Association and dissociation graph of 1:1 fitting model of Mouse CD8 aptamer#538 (biotinylated) to Mouse CD8 protein concentrations 500, 250 and 125 nM, by single reference method.

Table 1. K_d , R^2 and χ^2 values by Global fitting for single reference method. $K_d = 9.31 \text{ nM}$

Immobilized Aptamer	Analyte	Conc. (nM)	Response	K_d (M)	Full χ^2	Full R^2
Mouse CD8_#538_biotin	Mouse CD8 protein	500	0.2967	9.31E-09	0.063504	0.998578
Mouse CD8_#538_biotin	Mouse CD8 protein	250	0.0706	9.31E-09	0.063504	0.998578
Mouse CD8_#538_biotin	Mouse CD8 protein	125	0.008	9.31E-09	0.063504	0.998578

1e. Double reference data with immobilized random aptamer:

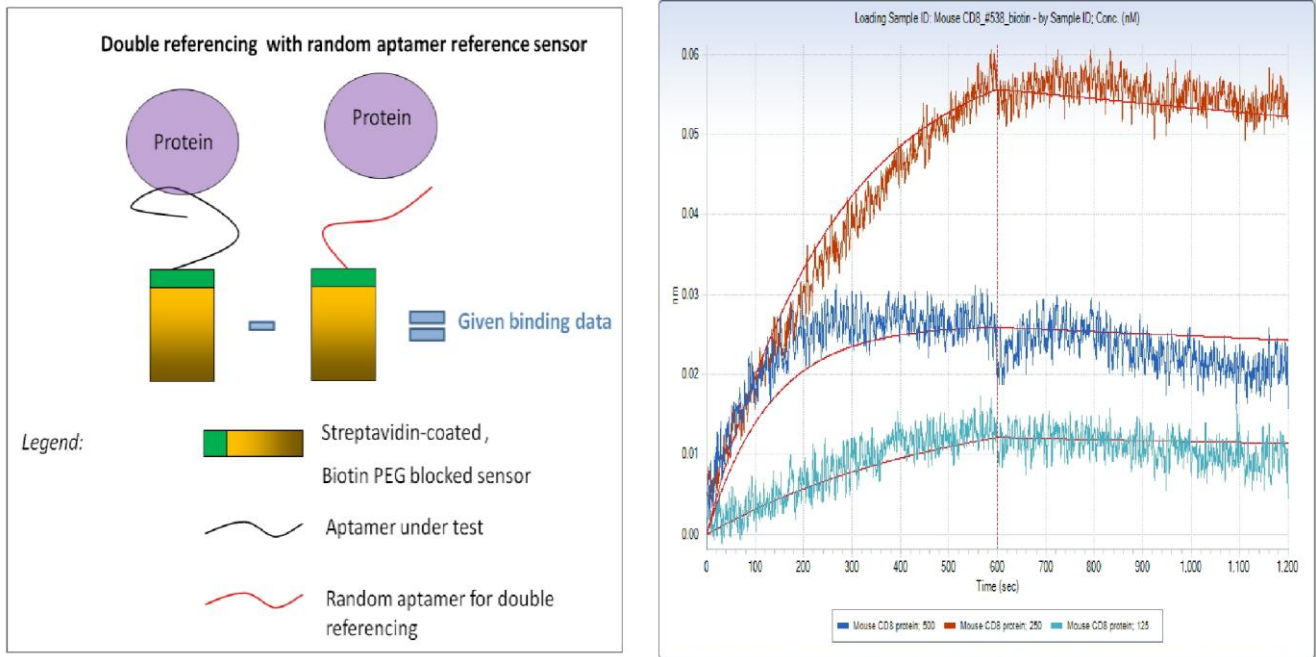


Figure 2. Association and dissociation graph of 1:1 fitting model of Mouse CD8 aptamer #538 (biotinylated) to Mouse CD8 protein concentrations 500, 250 and 125 nM, by double reference method with negative control aptamer.

Table 2. K_d , R^2 and χ^2 values by Global fitting for single reference method. $K_d = 7.14$ nM

Immobilized Aptamer	Analyte	Conc. (nM)	Response	K_d (M)	Full χ^2	Full R^2
Mouse CD8_#538_biotin	Mouse CD8 protein	500	0.0262	7.14E-09	0.026458	0.975154
Mouse CD8_#538_biotin	Mouse CD8 protein	250	0.0582	7.14E-09	0.026458	0.975154
Mouse CD8_#538_biotin	Mouse CD8 protein	125	0.0136	7.14E-09	0.026458	0.975154

Summary:

By single reference method, $K_d = 9.31$ nM
By double reference method, $K_d = 7.14$ nM