

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:

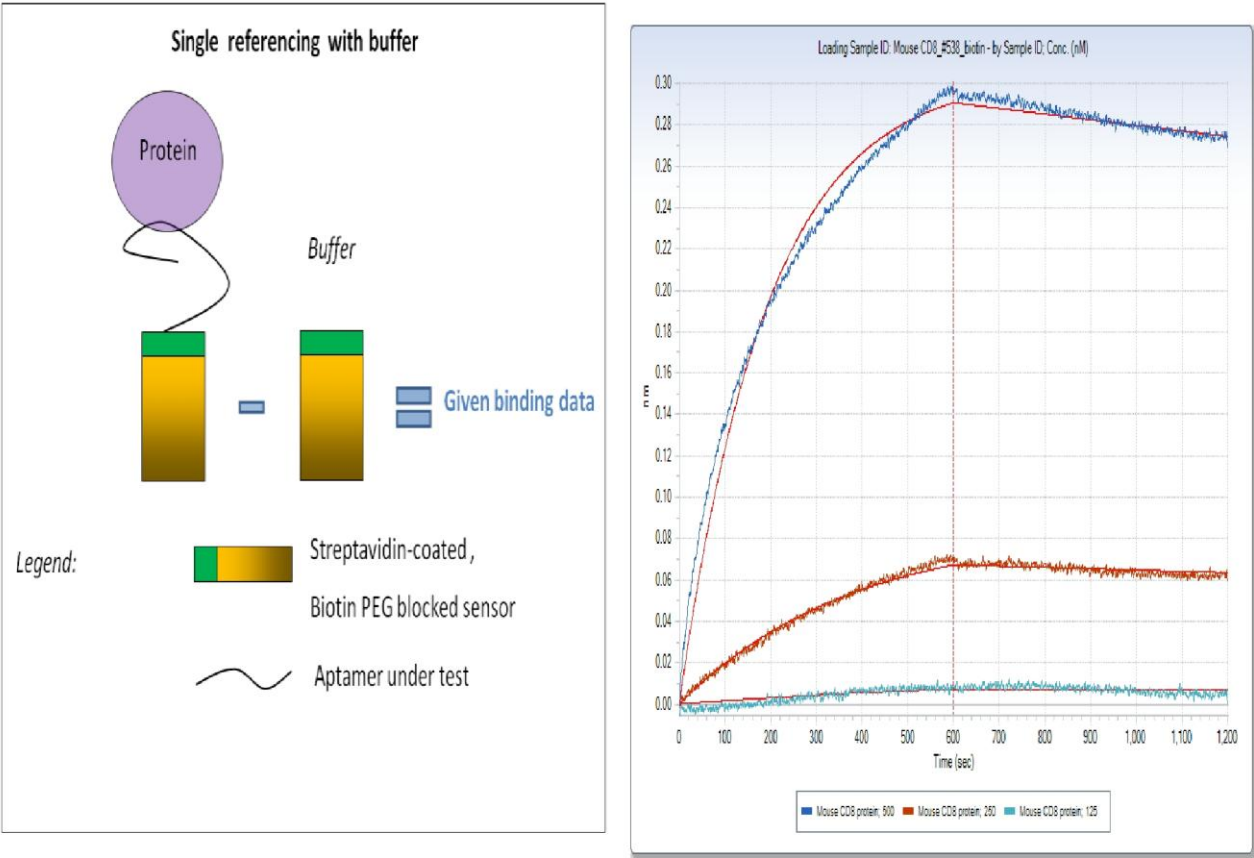


Figure 1. Association and dissociation graph of 1:1 fitting model of Mouse CD8 aptamer#538 (biotinylated) to Mouse CD8 protein concentrations 500, 250 and 125 nM, by single reference method.

| 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:

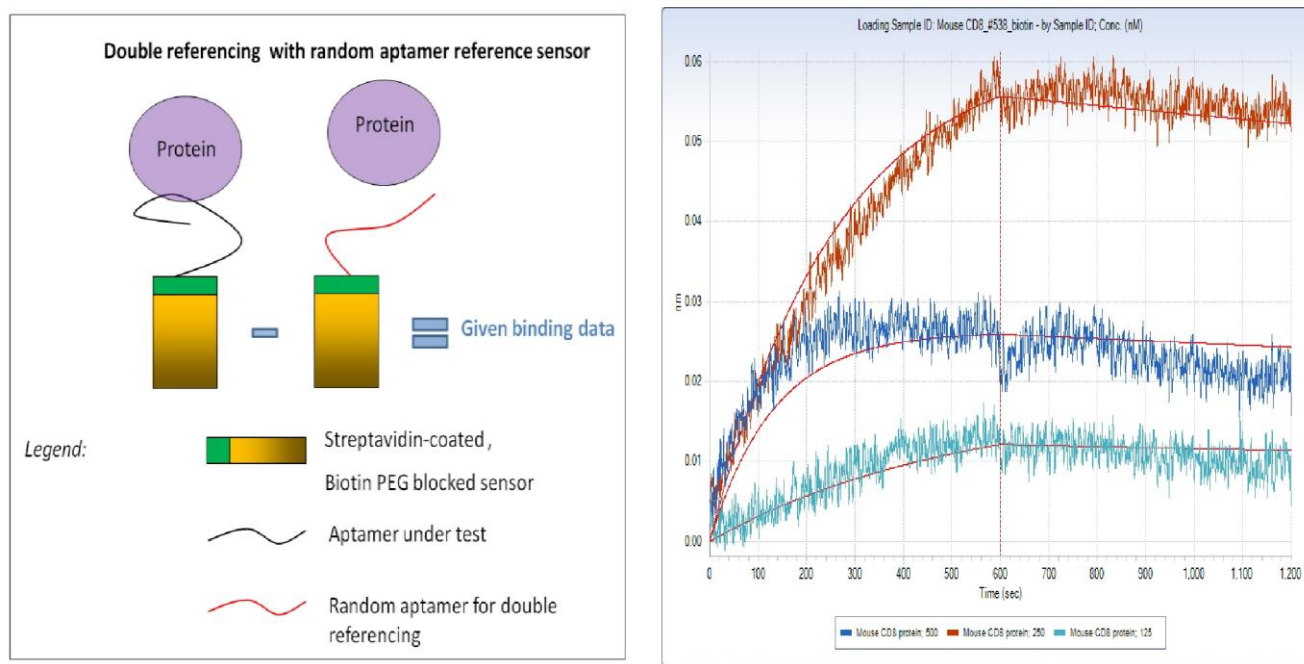


Figure 2. Association and dissociation graph of 1:1 fitting model of Mouse CD8 aptamer #538 (biotinylated) to Mouse CD8 protein concentrations 500, 250 and 125 nM, by double reference method with negative control aptamer.

| 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