



APTAMER INFORMATION

Tetanus aptamers Tet15 & Thj899

1. Tetanus aptamer Tet15

1a. Description:

- Identifiers: Tet15
- Number of DNA nucleotides: 70 bases
- Target for selection: Tetanus toxoid, List Biological Laboratories, Inc. (Cat. #191A)

Aptamer was selected from a randomized 32-mer library against the Tetanus toxoid 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 Tet15 by BSI (Back-Scattering Interferometry) method:

- Buffer used for validation: 1X PBS, pH = 7.4, 1 mM MgCl₂
- Average K_d = $15.9 \pm 9.0 \text{ nM}$

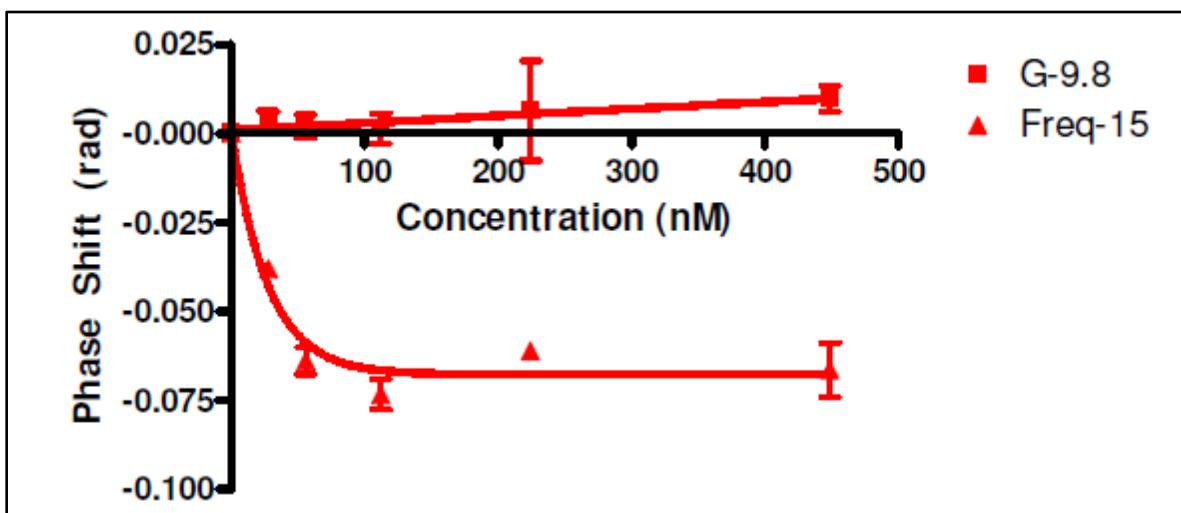


Figure 1. Aptamer-Tetanus toxoid binding. The phase shift of bound aptamer is plotted versus the titrated tetanus concentration. The values are the mean values from three independent measurements.



2. Tetanus aptamer Thj899

2a. Description:

- Identifiers: Thj899
- Number of DNA nucleotides: 32 bases
- Target for selection: **Tetanus toxoid, List Biological Laboratories, Inc. (Cat. # 191A)**

Aptamer was selected to form a sandwich pair with the Tet15- Tetanus toxoid protein complex. See Figure 2.

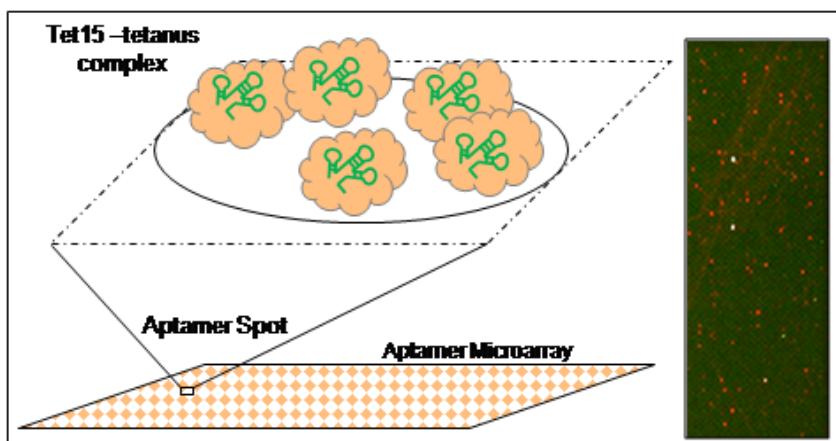


Figure 2. Sandwich-array assay. The labeled Tet15-Tetanus toxoid protein complex was pre-incubated together then introduced to an aptamer microarray in an effort to discover a sandwich pair for this target. After binding the Tet15- Tetanus complex to the microarray, the array was washed and scanned for fluorescence. Bound aptamers subjected to MST analysis.

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 Thj899 by MST (Microscale Thermophoresis) method:

- Buffer used for validation: 1X PBS, pH = 7.4, 1 mM MgCl₂
- Average K_d = $1.04 \pm 0.17 \text{nM}$

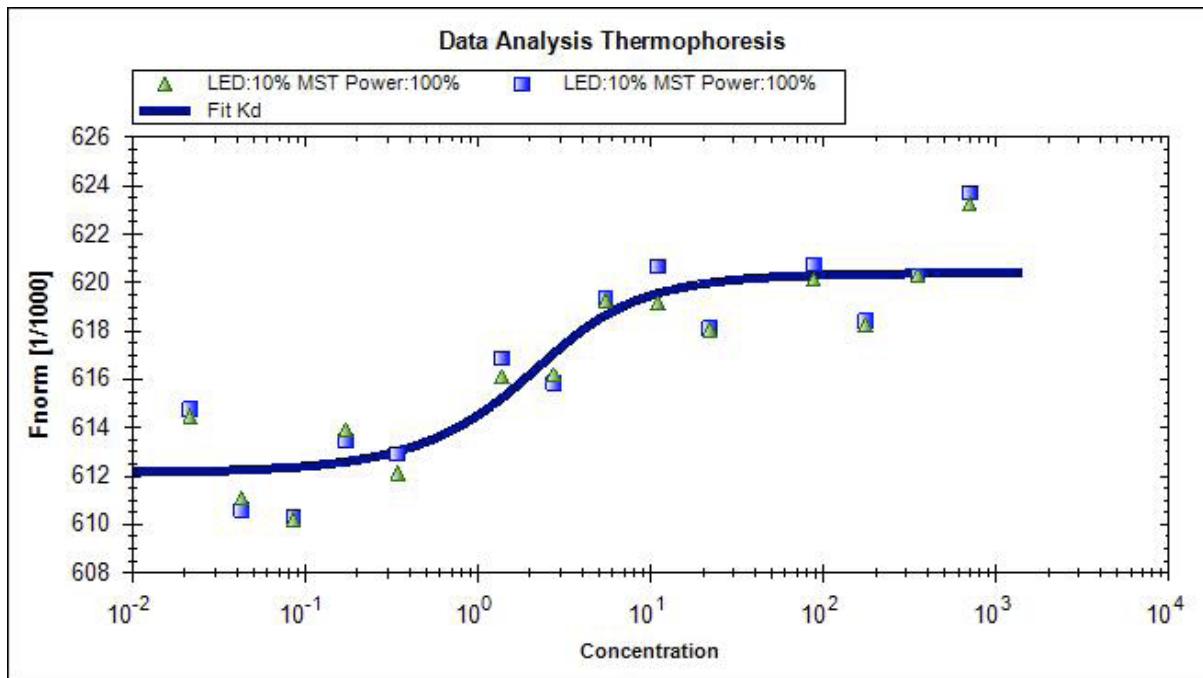


Figure 3. Experimental replicates 1 & 2. Aptamer-Tetanus toxoid binding. The thermophoresis is plotted versus the titrated protein concentration.