



APTAMER INFORMATION

Hemoglobin aptamer 72H09 #411

1a. Description:

- Identifiers: 72H09 (Oligo# 411)
- Number of DNA nucleotides: 40 bases (without 3'-5T), 45 bases (with 3'-5T)
- Molecular weight (includes 3'-5T and biotin): 14,256.4 g/mol
- Target for selection: Hemoglobin protein, Sigma (Part No. H7379).

Aptamer was selected from a randomized 40-mer library against Hemoglobin 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 Hemoglobin protein by BLI (Bio-Layer Interference) method:

- Immobilized Ligand: Hemoglobin aptamer 72H09 with 3'-5T and biotin
- Analyte: Hemoglobin protein
- Buffer used for validation: 20 mM Tris, 100 mM NaCl, 0.005% Tween20 in nuclease free water, pH 7.4
- Equilibrium Binding Analysis, Avg $K_d = 7.7$ nM

1c. Kinetics Screening Assay using Streptavidin Biosensors :

We validate the binding data by single reference method.

- Single reference data: All curves are referenced to a sensor dipped in buffer alone (no protein) (see Figures 1 & 2).



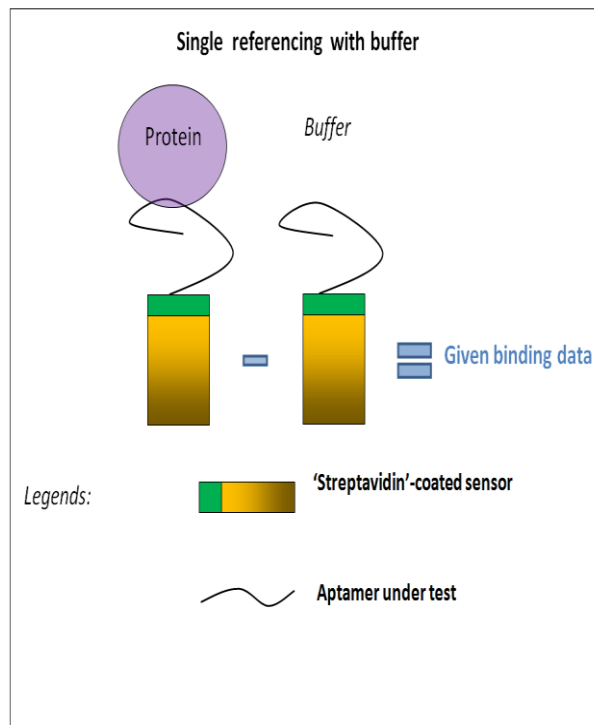


Figure 1. Diagram showing aptamer: protein binding validation scheme.



1d. Single reference data:

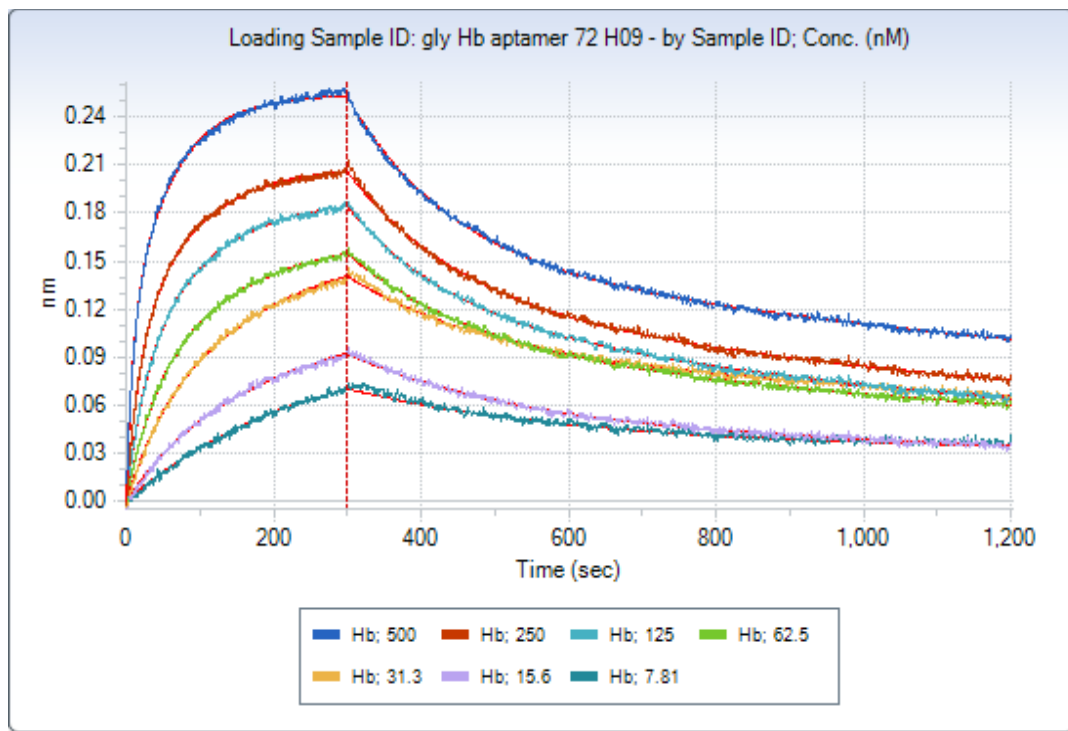


Figure 2. Association and dissociation graph of 2:1 (heterogenous ligand) fitting model of Hemoglobin aptamer 72 H09 (biotinylated) to Hemoglobin protein concentrations 500, 250, 125, 62.5, 31.3, 15.6, 7.81 nM by single reference method.

