

APTAMER INFORMATION

Crystallin AB aptamer #347

1a. Description:

- ***Identifiers:*** Oligo# 347
- ***Number of DNA nucleotides:*** 38 bases (without 3'-12T), 50 bases (with 3'-12T)
- ***Molecular weight (including 3'-12T and biotin):*** 15,783.4 g/mol
- ***Target for selection:*** Crystallin AB, Mouse Novus Biologicals (Cat #NBC1-18352)

Aptamer was selected from a randomized 40-mer library against Fibronectin 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 Crystallin AB protein by BLI (Bio-Layer Interferometry) method::

- ***Immobilized Ligand:*** Crystallin AB aptamer #347 with 3'-12T and biotin
- ***Analyte:*** Crystallin AB protein
- ***Buffer used for validation:*** 20 mM Tris, 100 mM NaCl, 0.005% Tween20 in nuclease free water, pH 7.4

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 and Table 1).

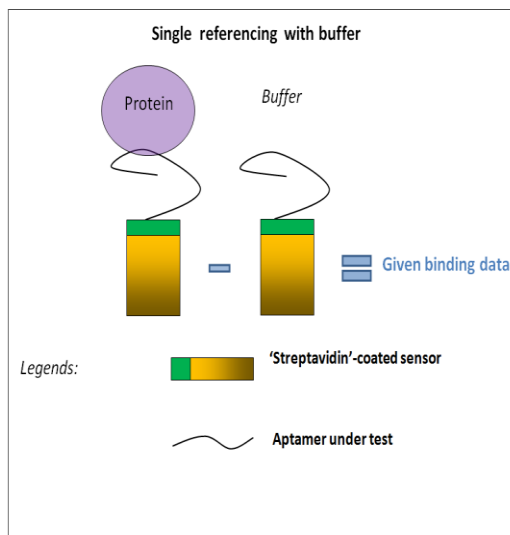


Figure 1. Diagram showing aptamer: protein binding validation scheme.

1d. Single reference data:

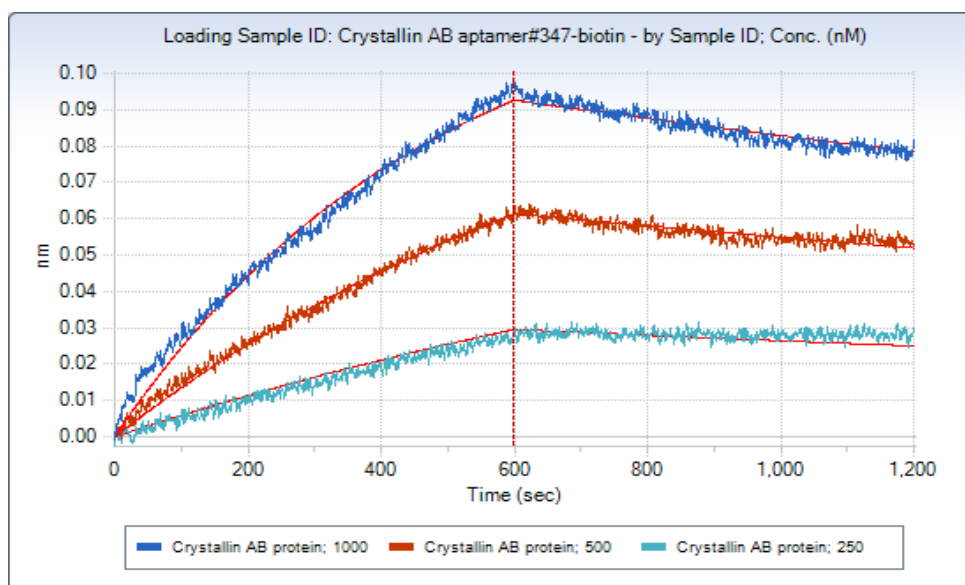


Figure 2. Association and dissociation graph of 1:1 fitting model of Crystallin AB aptamer #347 to Crystallin AB protein concentrations 1000, 500 and 250 nM, by single reference method.



Table 1. K_d , R^2 and χ^2 values by Local fitting for single reference method. **Avg K_d = 152 nM**

| Immobilized Aptamer | Analyte | Conc. (nM) | Response | K_d (M) | Full χ^2 | Full R^2 |
|-----------------------------------|---------------|------------|----------|-----------|---------------|------------|
| Crystallin AB Biotin aptamer #347 | Crystallin AB | 1000 | 0.0953 | 1.52E-07 | 0.012976 | 0.994903 |
| Crystallin AB Biotin aptamer #347 | Crystallin AB | 500 | 0.0609 | 1.52E-07 | 0.012976 | 0.994903 |
| Crystallin AB Biotin aptamer #347 | Crystallin AB | 250 | 0.0271 | 1.52E-07 | 0.012976 | 0.994903 |

