



APTAMER INFORMATION

CMVpp65_G2PVJ:1035:858_Oligo #757

Description:

- Identifiers: G2PVJ:1035:858 (oligo #757)
- Number of DNA nucleotides: 32 bases
- Molecular weight (includes 5'-bioTEG): 10,387g/mol
- Target for selection: **CMV pp65 protein**

Aptamer was selected from a randomized 32-mer library against CMV pp65 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 degC for 2 minutes, and then cooled to room temperature before use.

1. Validation data with CMVpp65 protein:

- Immobilized Ligand: Biotinylated CMVpp65 aptamer #757
- Analyte: CMVpp65 protein
- Random aptamer as negative control:
Random aptamer with same length, similar molecular weight and same 3' modifications (i.e 5'bioTEG) like CMVpp65 aptamer#757

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, 2 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 ~32-mer controls for any non-specific charge-only interactions.

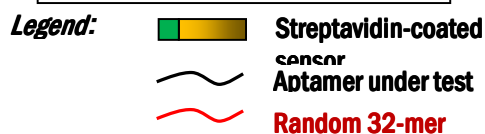
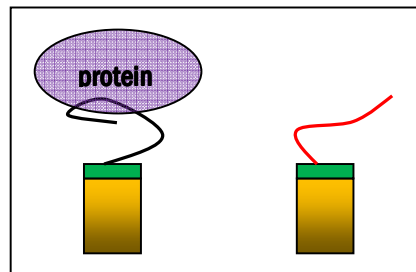




Figure 1: Double-referencing for highly stringent validation of aptamer: protein binding.
(i) Single reference data:

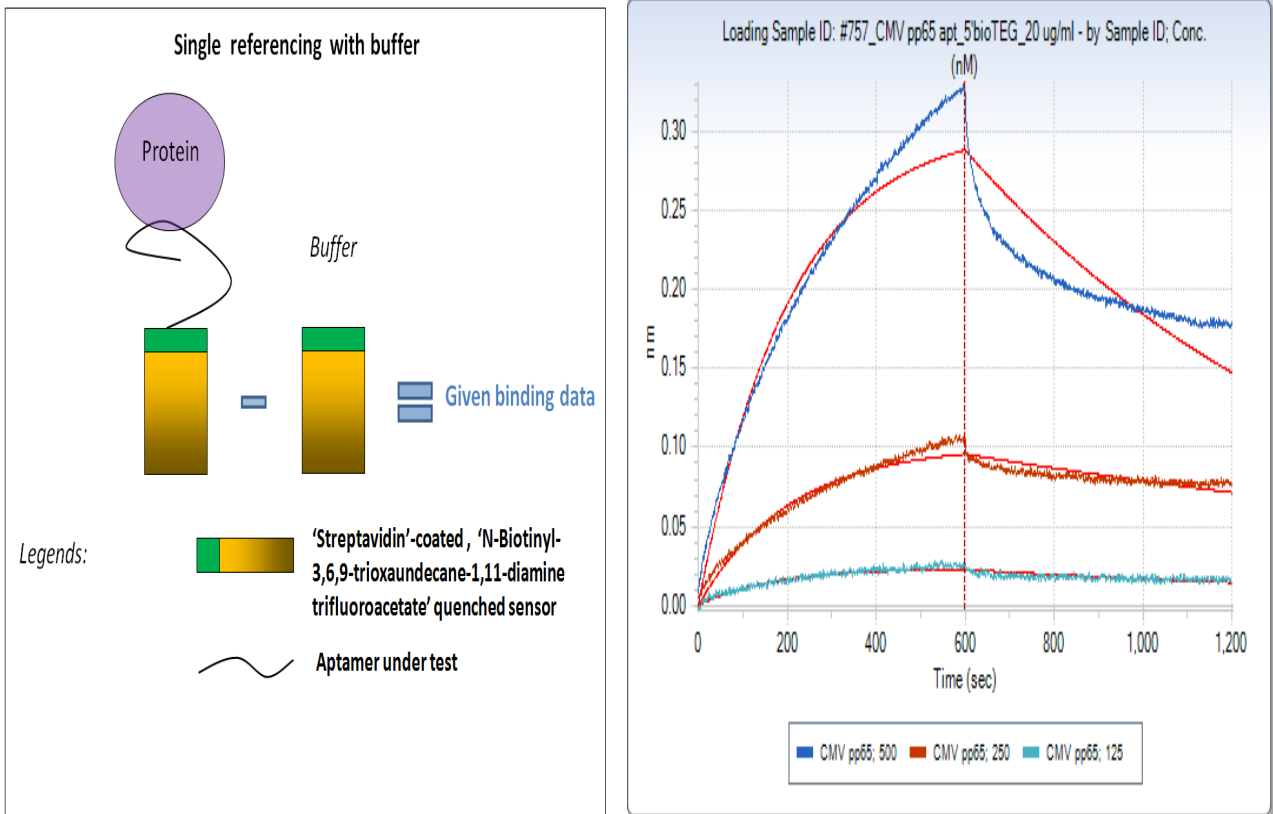


Figure 2. Association and dissociation graph of 1:1 fitting model of CMVpp65 aptamer # 757 (biotinylated) to CMVpp65 protein concentrations 500, 250 and 125 nM, by single reference method.

Table 1: Kd, R² and Chi² values by Local fitting for single reference method. **Avg Kd = 64 nM**

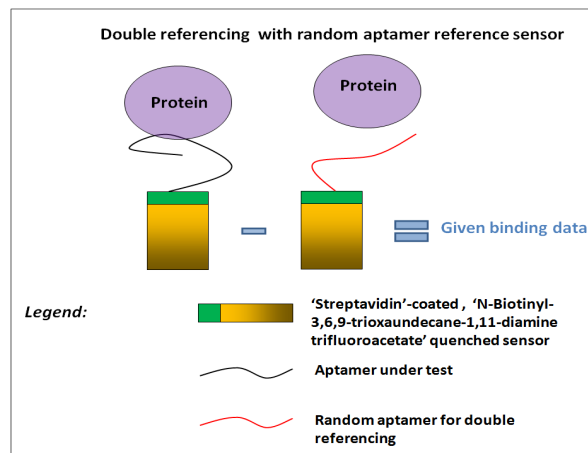
| Immobilized Aptamer | Analyte | Conc. (nM) | Response | KD (M) | Full X ² | Full R ² |
|-------------------------------------|----------|------------|----------|----------|---------------------|---------------------|
| #757_CMV pp65 apt_5'bioTEG_20 ug/ml | CMV pp65 | 500 | 0.3262 | 1.47E-07 | 0.414949 | 0.914212 |
| #757_CMV pp65 apt_5'bioTEG_20 ug/ml | CMV pp65 | 250 | 0.1053 | 2.80E-08 | 0.021597 | 0.955026 |





| | | | | | | |
|---|----------|-----|--------|----------|----------|----------|
| #757_CMV pp65 apt_5'bioTEG_20 ug/ml | CMV pp65 | 125 | 0.0252 | 1.73E-08 | 0.003901 | 0.859368 |
|---|----------|-----|--------|----------|----------|----------|

(ii) Double reference data with 'random aptamer immobilized sensors as reference sensors':



a) Highest conc. excluded in double reference data

b) Highest conc. included in double reference data



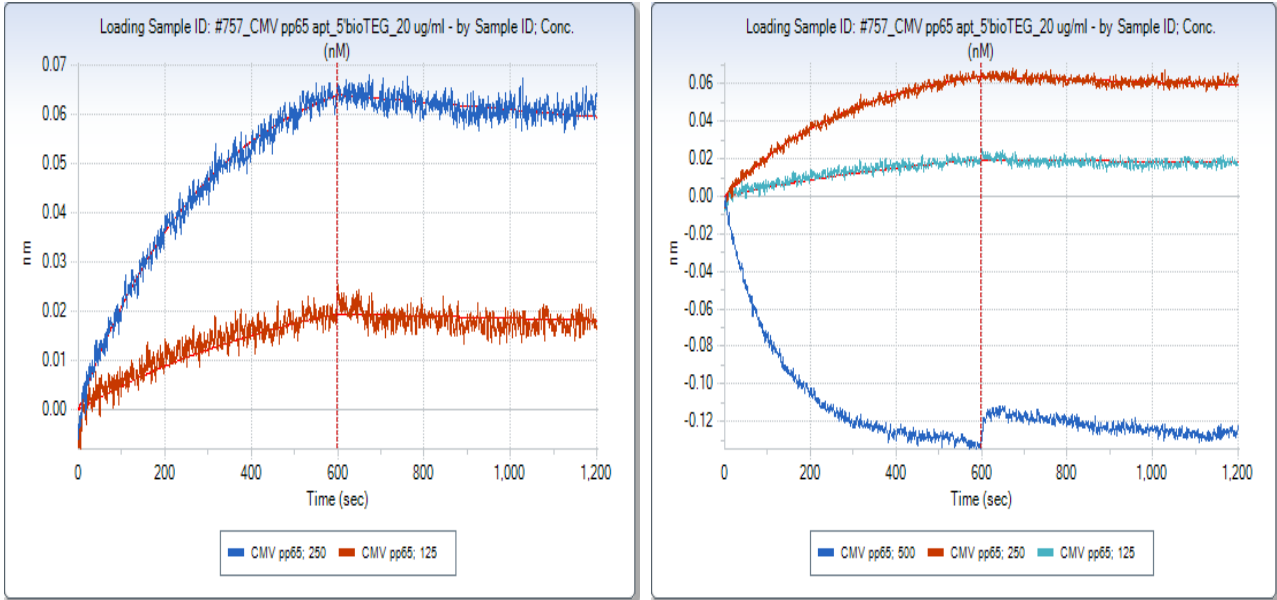


Figure 3. Association and dissociation graph of 1:1 fitting model of CMVpp65 aptamer # 757 (biotinylated) to CMVpp65 protein concentrations 500, 250 and 125 nM, by double reference method with ‘random aptamer immobilized and sensors as reference sensors’.

Table 2: Kd, R² and Chi² values by Global fitting for double reference method with ‘random aptamer immobilized and sensors as reference sensors’. **Kd = 9.31 nM**

| Immobilized Aptamer | Analyte | Conc. (nM) | Response | KD (M) | Full X ² | Full R ² |
|-------------------------------------|----------|------------|----------|----------|---------------------|---------------------|
| #757_CMV pp65 apt_5'bioTEG_20 ug/ml | CMV pp65 | 250 | 0.0628 | 9.31E-09 | 0.008206 | 0.992869 |
| #757_CMV pp65 apt_5'bioTEG_20 ug/ml | CMV pp65 | 125 | 0.0192 | 9.31E-09 | 0.008206 | 0.992869 |

