

Octet[®] Anti-Penta-HIS (HIS1K) Biosensors

For Label-Free Quantitation
and Kinetic Analysis of
His-tagged Proteins

Key Features

- High specificity quantitation of His-tagged proteins
- Easy capture of His-tagged proteins for kinetic analysis with interacting protein analyte
- High specificity and high affinity towards polyhistidine tag
- Allows rapid analysis in purified or crude samples



Overview

The polyhistidine tag, commonly known as His-tag, is fused to recombinant proteins as a means of facilitating detection and purification. The Dip and Read Anti-Penta-HIS (HIS1K) Biosensor consists of high affinity, high specificity Penta-His antibody from QIAGEN pre-immobilized on a Sartorius fiber optic biosensor. In conjunction with the Octet[®] systems, the HIS1K Biosensor provides a rapid and label-free method for His-tagged protein quantitation and kinetic analysis. The high specificity of the antibody-based biosensor enables the direct capture and quantitation of His-tagged proteins in crude lysates, column eluates, cell lysates and cell culture supernatants, serving as an alternative to traditional time-consuming analytical methods such as HPLC and ELISA.

Flexibility and Versatility

The Anti-Penta-HIS Dip and Read Biosensor is qualified for both kinetic and quantitation applications. It enables users to quickly and easily detect His-tagged recombinant proteins for quantitation measurements, or to capture them for affinity measurements with interacting analytes. Together with the Octet® N1 system's ease of use or the Octet® platform's throughput, HIS1K Biosensors greatly accelerate laboratory workflows and reduce time to results. The Octet® N1 system further enables measurement of precious samples with sample volume requirements as low as 4 µL. The HIS1K Biosensor can be regenerated for kinetic applications, providing a cost-effective solution for generating replicate data for ligand-analyte pairs, or for analyzing multiple analytes.

Range of Applications

The Anti-Penta-HIS Biosensor offers researchers unparalleled ease of use and time-to-results in a wide range of laboratory applications such as:

- Rapid quantitation of any His-tagged protein
- Protein expression monitoring
- Cell line development/optimization

Affinity characterization of interactions between His-tagged proteins and biomolecular analyte binding partners

For technical information on the Anti-Penta-HIS (HIS1K) Biosensor, see Technical Note 42 (*Anti-Penta-HIS (HIS1K) Biosensors for Label-Free Analysis of His-tagged Proteins*).

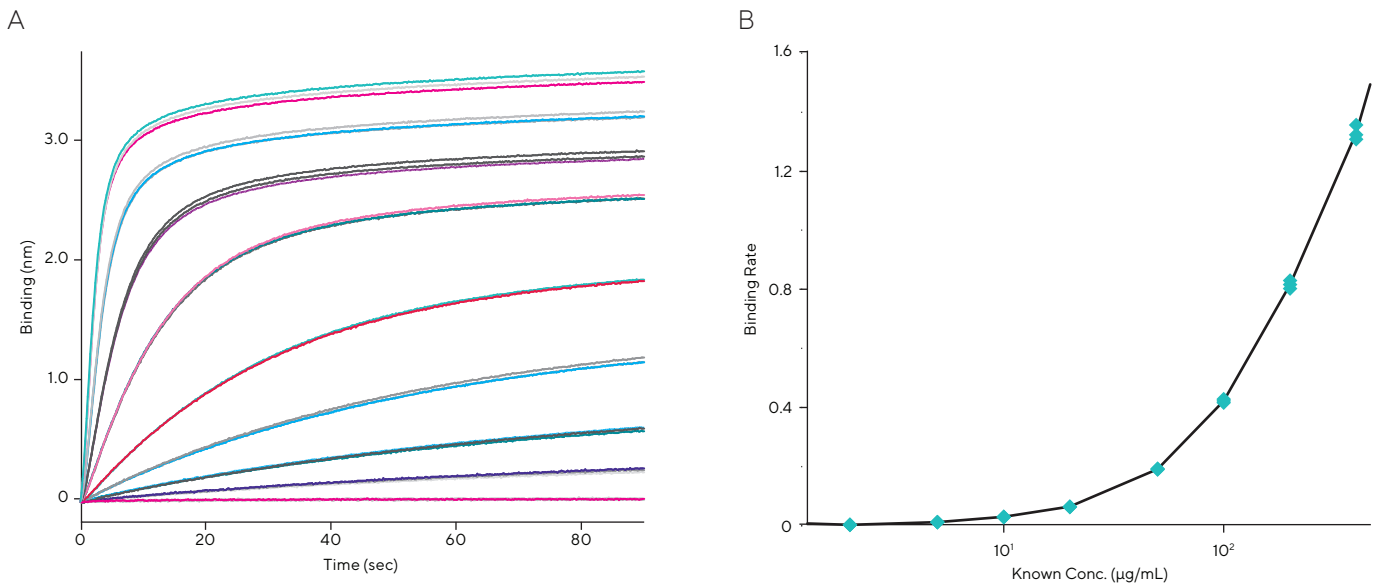


Figure 1: Detection of His-PAI1 and His-Protein A standards using Anti-Penta-HIS Biosensors on the Octet® RH16 system. (A) Raw data, (n=3). (B) Calibration curve for His-PAI1. Sample diluent was used as matrix for all samples, assay run at 1000 rpm.

Table 1: Average calculated concentration and %CV of triplicates of His-PAI1 calibration standards for the data from Figure 1. Results may vary with individual His-tagged analytes and assay matrices.

| Known conc. (µg/mL) n=3 | Average binding rate | Average calculated concentration | % Recovery | %CV |
|----------------------------|----------------------|----------------------------------|------------|-------|
| 400 | 1.3267 | 400.0 | 100.0% | 1.8% |
| 200 | 0.8136 | 200.6 | 100.3% | 2.6% |
| 100 | 0.4211 | 100.1 | 100.1% | 2.1% |
| 50 | 0.1911 | 50.0 | 99.9% | 3.3% |
| 20 | 0.0635 | 20.0 | 99.8% | 3.1% |
| 10 | 0.0287 | 10.0 | 100.0% | 2.3% |
| 5 | 0.0117 | 5.0 | 99.9% | 3.5% |
| 2 | 0.0029 | 2.0 | 98.8% | 14.1% |

Ordering Information

| Part No. | UOM | Description |
|----------|------|--|
| 18-5120 | Tray | One tray of 96 Octet® HIS1K Biosensors |
| 18-5121 | Pack | Five trays of 96 Octet® HIS1K Biosensors |
| 18-5122 | Case | Twenty trays of 96 Octet® HIS1K Biosensors |

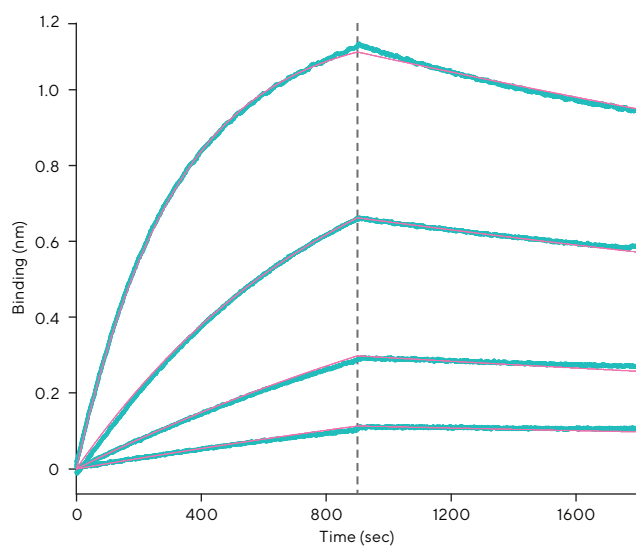


Figure 2: Kinetic analysis of the interaction between ligand His-tagged Rat C-Reactive Protein (24 kDa) and analyte Mouse Anti-Rat C-Reactive Protein (150 kDa). 10X Kinetics Buffer was used as the matrix throughout and the assay temperature was 30°C. Data were processed and curve fit using a 1:1 binding model. The kinetic results are reported in Table 2.

Table 2: Kinetic results for the interaction between ligand His-tagged Rat C-Reactive Protein (24 kDa) and analyte Mouse Anti-Rat C-Reactive Protein (150 kDa) using Anti-Penta-HIS Biosensors.

| K_D (M) | k_{on} (1/Ms) | k_{dis} (1/s) |
|-----------|-----------------|-----------------|
| 1.79E-09 | 9.25E+04 | 1.71E-04 |

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