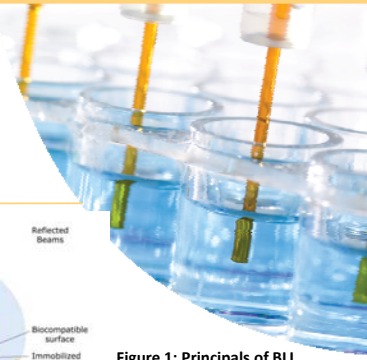


# New label-free direct detection method for HIS-tagged protein quantitation

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## Abstract

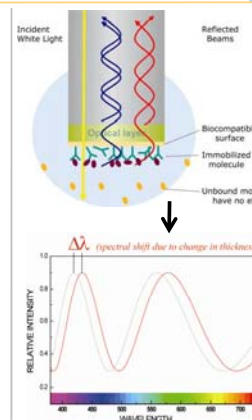
The penta-histidine tag (HIS-tag) is a common peptide tag fused to recombinant proteins during cloning. Many of the common techniques for detecting these recombinant proteins require both a capture antibody directed against the HIS-tag and a detection antibody directed against the protein itself, which may not always be available. In the experiments described here, we used biolayer-interferometry technology and a specific anti-Penta-HIS antibody pre-immobilized onto a biosensor surface to capture and quantify HIS-tag proteins in a label-free method that does not require any secondary detection antibody. Using this new method, we tested proteins with molecular weights ranging from 13 kDa up to 43 kDa and determined that the assay has a dynamic range of approximately 0.25-200 micrograms per milliliter in both purified form and spiked into spent CHO cell media. In both scenarios the assay provided very comparable results demonstrating that this new assay provides a very rapid and easy method for quantitation of HIS-tag proteins in both purified and crude media.

## Materials

The experiments described here were performed using the following materials and instruments:

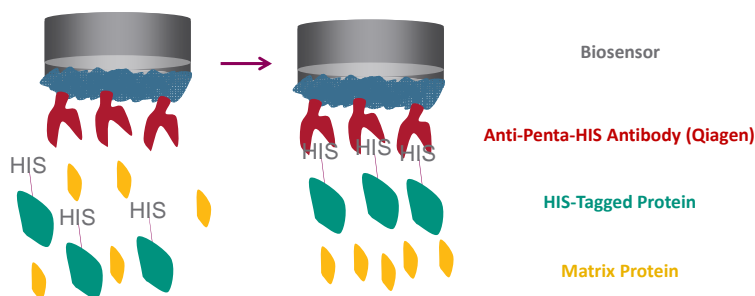
- Anti-Penta-HIS (HIS) Biosensors (ForteBio P/N 18-5077)
- Sample Diluent (ForteBio P/N 18-5028)
- Octet™ QK and Octet RED Instruments
- HIS-tagged Human Resistin (R&D Systems P/N 841180)
- HIS-tagged PAI (Oxford Biomedical Research P/N PI06)

The Octet platform of instruments uses biolayer interferometry technology to detect and quantify biomolecular interactions (see Figure 1 and [fortebio.com](http://www.fortebio.com) for a full explanation of the detection technology). The method used to prepare and analyze the samples described here is described in detail in Technical Note number 19 "Anti-Penta-HIS Biosensors" available at [www.fortebio.com/literature](http://www.fortebio.com/literature).



**Figure 1: Principles of BLI**  
Octet provides real-time monitoring for protein:protein interactions and binding events using Biolayer Interferometry (BLI) technology. Any change in the number of molecules bound to the biosensor tip changes the optical layer thickness which causes a shift in the interference pattern ( $\Delta\lambda$ ). As these shifts are monitored over time, a real time binding curve is generated.

## Quantitation of HIS -tagged Proteins

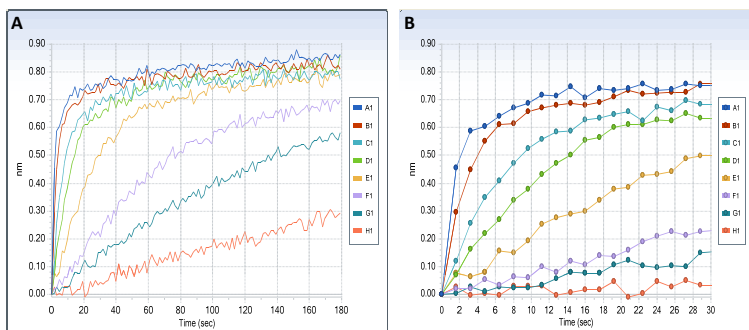


**Figure 2: Quantitation of HIS-tagged protein assay description and protocol**

Anti-Penta-HIS biosensors coated with the Anti-Penta-HIS antibody from Qiagen (P/N 34660). Using these biosensors, HIS-tagged proteins can be captured directly from purified or crude samples using the following protocol.

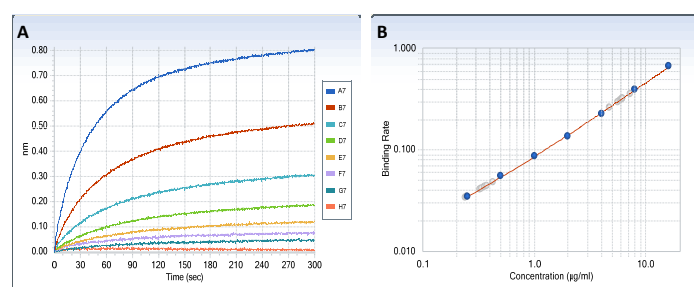
1. Dilute samples into appropriate matching matrix
2. Load 200 $\mu$ L of each sample into microplate
3. Pre-wet biosensors in blank sample matrix
4. Run assay on Octet system to monitor and measure molecules binding the biosensor (3-5 min/column; 40 minutes total assay time on Octet; 20 minutes total assay time on Octet 384)
5. Analyze data using Octet data analysis software using either Initial slope or R equilibrium binding rate equation (v6.1 or later)

## Experimental Results



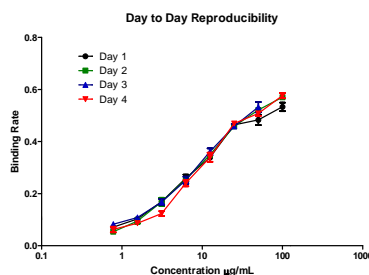
**Figure 3: Quantitation of HIS-tagged Resistin**

A) Binding data from a concentration series of HIS-tagged recombinant human Resistin (13 kDa) diluted in Sample Diluent, obtained using the Anti-Penta-HIS biosensor on an Octet QK. Concentrations for each channel ( $\mu$ g/mL): A1= 50; B1 = 25; C1 = 10; D1 = 5; E1 = 2.5; F1 = 1; G1 = 0.5; H1 = 0.2. Note that the higher concentration binding curves (5–50  $\mu$ g/mL) have a similar nanometer (nm) shift at the end of the assay. B) Enlargement of the first 30 seconds of the binding data from Figure 3A. Note that the initial binding rates differ greatly, indicating that an analysis using the Initial Slope algorithm is preferred. C) Calibration curve generated using the Initial Slope binding rate equation.



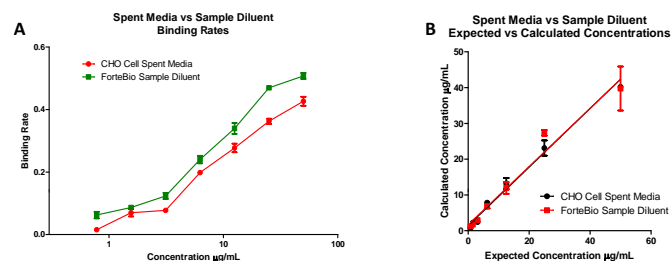
**Figure 4: Quantitation of HIS-tagged PAI**

A) Raw binding data for HIS-tagged PAI (43 kDa) diluted in Sample Diluent obtained using the Anti-Penta-HIS biosensor on an Octet RED. Concentrations for each channel ( $\mu$ g/mL): A1= 16; B1 = 8; C1 = 4; D1 = 2; E1 = 1; F1 = 0.5; G1 = 0.25; H1 = 0. Note the large difference in the final nm shift at the end of the 5-minute assay, indicating that using the R Equilibrium algorithm would be preferred. B) Calibration curve generated from binding data for HIS-tagged PAI using the R Equilibrium algorithm.



**Figure 5: Reproducibility of quantitation of HIS-tagged proteins**

In this example, a 40 kDa protein was diluted in Sample Diluent from 100  $\mu$ g/mL to 0.78  $\mu$ g/mL and run in the Octet QK at 1000 rpm for 5 minutes per column. The curve was run on 4 independent days (day 3 had a maximum concentration of 50  $\mu$ g/mL). All data from each day was analyzed using the R equilibrium algorithm. All concentrations had a % CV < 15%.



**Figure 6: Quantitation of HIS-tagged proteins in spent CHO cell media**

The 40 kDa protein from Figure 5 was diluted in both Sample Diluent and mammalian CHO cell spent media from 50  $\mu$ g/mL to 0.78  $\mu$ g/mL and run in the Octet QK at 1000 rpm for 5 minutes per column. Note that while total signal in the Sample Diluent is slightly better (A), the calculated concentrations are nearly identical in either media (B).

## Summary

- Quantitation using the Octet system and the Anti-Penta-HIS biosensor provides a direct and rapid means of quantifying HIS-tagged proteins without requiring any secondary amplification reagents.
- The protocol is very simple and involves no wash steps.
- Flexible data analysis options accommodate multiple binding curve shapes for accurate determination of protein concentration.
- Quantitation of HIS-tagged proteins using the Octet system is accurate and reproducible in both standard buffers and crude cell supernatants.

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