

Anti-Penta-HIS (HIS) Biosensors

Label-Free Quantitation of HIS-Tagged Proteins

Key Features

- Quantitate proteins containing a six-histidine or penta-histidine peptide tag (HIS-tag)
- Designed for use in buffer, complex media, column eluates, and cell lysates
- Binds HIS-tagged proteins even in the presence of imidazole
- Easy to use, label-free, no wash protocol

INTRODUCTION

The six-histidine or penta-histidine peptide tag (HIS-tag) is a common peptide tag fused to recombinant proteins during cloning. Many tools have been developed enabling the tag to be used for easy detection and purification of tagged proteins. The Anti-Penta-HIS biosensor, for use on the Octet system, now provides a rapid, label-free method for quantification of HIS-tagged proteins. This biosensor comes with the highly specific Qiagen Penta-HIS antibody pre-immobilized on the surface and is ready to use for specific detection and quantitation of HIS-tagged proteins.

QUICK FACTS

- **Immobilization Chemistry:** highly specific Qiagen Penta-HIS antibody (Qiagen part no. 34660)
- **Quantitation Dynamic Range:** 0.25–200 µg/mL
- **Compatible Matrix Types:** buffers, complex media, column eluates, and cell lysates

INTENDED USE

The biosensor is intended for the detection and quantitation of HIS-tagged proteins. Proteins can be purified, in column eluates,

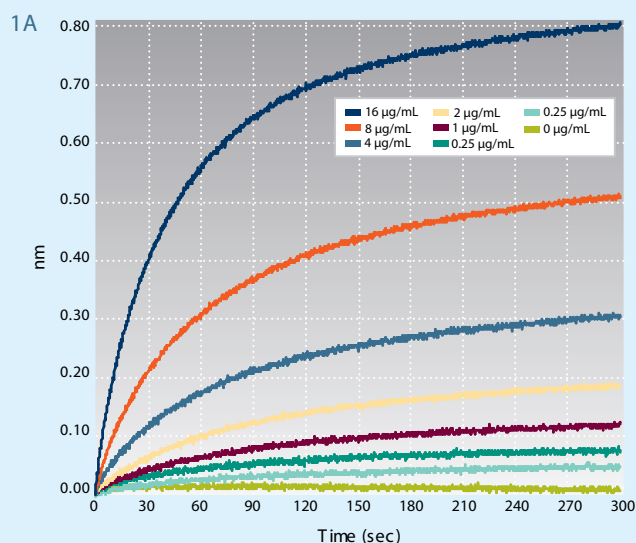


FIGURE 1A: Real-time binding data from a concentration series of HIS-tagged PAI (Oxford Biomedical Research, part no. PI06) obtained using the Anti-Penta-HIS biosensor on an Octet RED. The concentration series tested was 16, 8, 4, 2, 1, 0.5, 0.25 and 0 µg/mL.

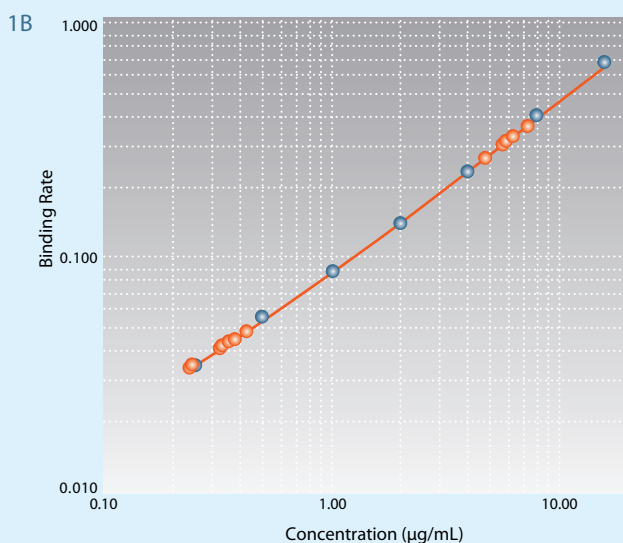


FIGURE 1B: Calibration curve generated from binding data for HIS-tagged PAI using the R Equilibrium algorithm.

Sample Type	Minimum Recommended Dilution In ForteBio Sample Diluent
Purified proteins	Dilute into assay range as necessary
Samples containing imidazole (elutes from NTA resin)	Dilute into assay range as necessary
Serum-free cell culture supernatants media	1:10 dilution
Serum-containing cell culture supernatants	1:100 dilution
Bacterial cell pellet lysed by freeze-thaw	1:10 dilution
Bacterial cell pellet lysed by detergent	1:100 dilution

TABLE 1: Recommended minimum dilution for each sample type. NOTE: In all cases the matrix for the diluted samples, the calibrator and the biosensor hydration solution should be matched as closely as possible.

cell culture supernatants or cell lysates. Performance in each matrix type should be optimized through appropriate sample dilution and careful construction of a suitable calibration curve. This biosensor is intended for research and manufacturing use only and is not intended for diagnostic use in humans or animals.

APPLICATIONS

The Qiagen Penta-HIS antibody (Qiagen part no. 34660) is preimmobilized onto the biosensors and will bind specifically to a HIS-tag attached to recombinant proteins (refer to the Qiagen *QIAexpress Handbook* for more specific epitope information). This binding is monitored in real time using the Octet system and can be compared to the binding of a known calibrator to determine concentration (Figure 1). Since the antibody-coated biosensors will bind HIS-tagged protein in the presence of complex matrix components or imidazole, the Anti-Penta-HIS biosensor can be used to quantitate HIS-tagged proteins from Ni/NTA column elutes, cell culture supernatants (Figure 2) and cell lysates after the appropriate dilution into sample diluent (Table 1).

TYPICAL ASSAY PARAMETERS

- **Sample Volume:** 200 μ L/well (post-dilution)
- **Hydration Sample Volume:** 200 μ L/well (post-dilution)
- **Flow rate:** 1000 rpm
- **Biosensor Hydration and Sample Plate Equilibration:** 10 minutes
- **Assay Time per Column of Samples:** 3–5 minutes

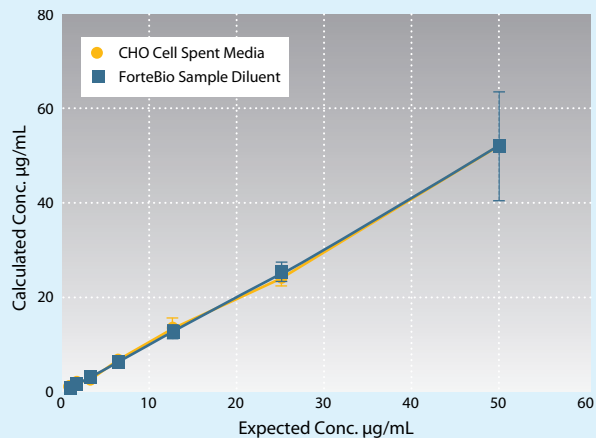


FIGURE 2: Comparison of calculated concentrations for the quantitation of the same HIS-tagged protein in ForteBio Sample Diluent and CHO cell spent media.

ORDERING INFORMATION

Part No.	UOM	Description
18-5077	Tray	One tray of 96 Anti-Penta-HIS (HIS) biosensors
18-5078	Pack	Five trays of 96 Anti-Penta-HIS (HIS) biosensors
18-5079	Case	Twenty trays of 96 Anti-Penta-HIS (HIS) biosensors

Note: additional materials are required to run these assays. Please consult Technical Note 19 for full details.

Dip and Read Anti-Penta-HIS biosensors are compatible with all Octet instruments including the new Octet 384 series. The latest version of software includes a predefined protocol to make this assay quick and easy to run and is available with 21 CFR Part 11 compliance tools.

For more information about ForteBio's Octet platform for label-free, real-time detection of biomolecular interactions, applications, and services, visit www.fortebio.com or contact us directly.