



Direct quantification of His-tagged proteins in solution

His-tag is widely used to aid recombinant protein purification and detection. Currently, detection of recombinant His-tagged proteins involves laborious procedures such as gel electrophoresis. BioXene's His-tag detection sensor reagent is superior to existing products, allowing researchers and practitioners alike a much quicker, simpler method of not only detecting but also quantifying His-tagged recombinant proteins directly in solution, without running gel electrophoresis. Competing technologies for His-tagged protein detection typically take several hours and require multiple steps in the assay, not even counting the additional steps required for quantifying the concentration of the protein. BioXene's His-tag sensor technology reduces the entire assay process to less than half an hour in a simple mix and measure procedure. The ability of using BioXene's sensor proteins in homogeneous assays allows users to easily track and monitor the His-tag proteins in-solution with minimum sample preparation.

How it Works

The working principle of BioXene's novel self-reporting sensor platform, including the His-tag sensor system, is based on modulating the Förster resonance energy transfer (FRET) property of uniquely-designed sensor proteins upon binding to their targets. The sensor protein comprises a molecular (protein) scaffold, a pair of labels which interact *via* FRET, and one or more ligand/molecular recognition domain (MRD) grafted onto the protein scaffold or the FRET label by chemical conjugation or genetic fusion. These sensor proteins serve as efficient self-reporting optical transducers. When placed in contact with a target analyte, the sensor protein displays a change in its FRET property that can be conveniently detected using standard fluorometry. These sensor molecules are considered self-reporting since no additional reagent/label for signal generation is required. The modular nature of the sensor protein design provides a molecular platform that can be conveniently modified, by changing the MRD, for detecting different biomolecular binding events both *in vitro* and *in vivo*. The working principle of the sensor is depicted in Figure 1.

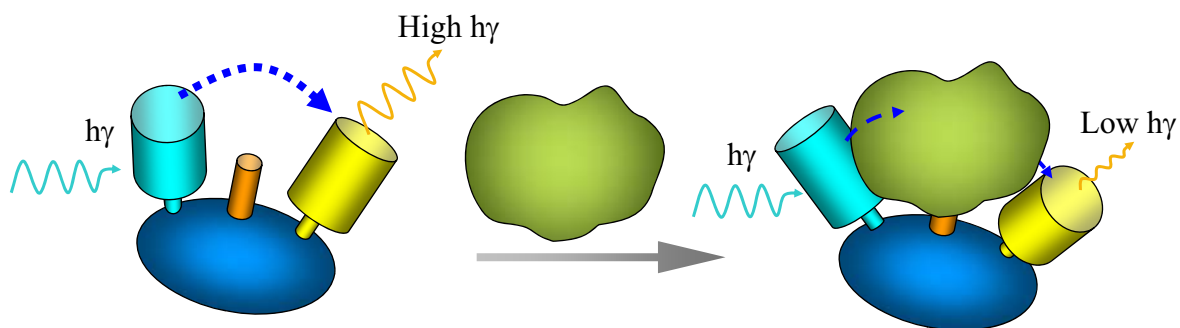


Figure 1. Sensor protein working principle.

Simplified Detection Process

- BioXene His-tag sensor protein is incubated directly with a sample in solution for 20 minutes and read by a fluorometer (no laborious sample preparation, labeling or washing is required).
- Measurements allow rapid quantification of His-tagged protein concentration in solution.
- Results are provided in less than half an hour, saving significant time and money.
- The sensor readout is expressed in terms of the FRET ratio, which is defined as the ratio of fluorescence intensities at the two emission peaks of the sensor, while the sensor is excited at the optimal donor excitation wavelength. The sensor response expressed as the normalized FRET ratio, Ψ , for a given target analyte concentration, A , and a sensor protein concentration, P , can be quantified to estimate the concentration of the target analyte in solution using a modified Scatchard equation (*i.e.* $A = (\Psi - 1) \left(\frac{P}{(\Psi_s - 1)} + \frac{K_D}{(\Psi_s - \Psi)} \right)$, where Ψ_s and K_D are the normalized FRET ratio at saturation and equilibrium dissociation constant, respectively).

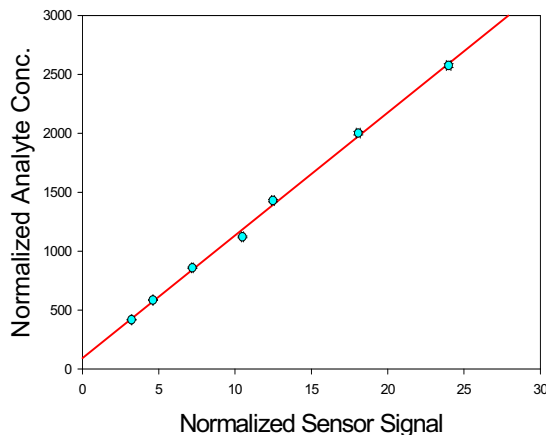


Figure 2. Direct quantification of His-tagged chloramphenicol acetyltransferase (CAT). The measurement covered CAT-His concentrations from 50 nM to 1 μ M using 50 nM of sensor protein. The normalized analyte concentration and normalized sensor signal represent $A/(1-\Psi)$ and $1/(\Psi-\Psi_s)$, respectively.

References

- Su, W.W. 2007. Sensor constructs and detection methods. US patent 7,247,443, issued on July 24, 2007.
- Vardar-Schara, G., Krab, I.M., Yi, G., Su, W.W. 2007. A homogeneous fluorometric assay platform based on novel synthetic proteins. *Biochem. Biophys. Res. Commun.* 361, 102-108.

BioXene™ is a Hawaii-based biotechnology company. Formed in 2004, BioXene is focused on developing state of the art biosensing technology for rapid and sensitive detection of a wide variety of targets. Utilizing a proprietary patented platform technology developed at the University of Hawaii, BioXene's unique sensor-protein technology offers several key distinctive competitive advantages over existing molecular detection methods such as ELISA and Western blot.

BioXene welcomes strategic collaboration and distribution partnerships as well as sponsors and investors who are interested in investing in BioXene.

Contact Information:

Victor Wong

BioXene

4224 Waiālae Avenue #276

Honolulu, HI 96816 USA

Tel: (808) 781-9537

Email: victor@bioxene.com

Website: <http://www.bioxene.com>