Easy assay setup and high-quality data using the LiquiChip™ System

The LiquiChip™ Protein Suspension Array System is a bead-based platform that enables a wide range of protein interaction arrays to be carried out quickly and with high sensitivity (see *QIAGEN News* 2002 No 2, 1). Here we show how assays using 6xHis-tagged proteins can be quickly and easily developed and used to obtain high-quality assay data.

Easy assay setup

One of the major advantages of the LiquiChip System is that it enables QIA*express*® 6xHis technology to be used in *x*MAP™ bead-based assays. This means that a single chemistry can be used for all steps of the protein-handling process, from purification to immobilization and assay. Using a common immobilization protocol for 6xHis-tagged proteins increases standardization in assays and eliminates the need for lengthy and cost-intensive optimization trials (Figure 1). Proteins are bound to beads in a batch procedure and in most cases can be added directly to LiquiChip Assays. Table 1 shows a comparison of the steps involved in coupling 6xHis-tagged proteins to LiquiChip Ni-NTA and Penta·His™ Beads with those required for covalent protein immobilization.

In addition to being quicker, immobilization of 6xHis-tagged proteins on Ni-NTA and Penta·His Beads requires less protein per assay point. Proteins are bound in a directed manner, maximizing accessibility to interaction partners and increasing signal intensity.

High-quality data sets

Assay data quality is reflected in signal-to noise and signal-to-background ratios. The Z-factor (see Table 2 and reference 1) is a dimensionless, simple statistical characteristic and the most suitable parameter for assay quality assessment. The Z-factor reflects assay signal dynamic range and variation associated with signal measurements.

To determine the Z-factor of a typical LiquiChip assay, a hypothetical drug-screening assay using proteases was established (Figure 2). 6xHis-tagged Thioredoxin–Tag·100 was immobilized on LiquiChip Ni-NTA Beads, incubated with differing amounts of enterokinase, and residual noncleaved protein was quantified after 3 hours incubation using Tag·100 specific antibodies. Data were collected from three processed 96-well plates (n = 288). ▶

Table 1. Steps involved in coupling 6xHis-tagged proteins to LiquiChip Ni-NTA and Penta·His Beads in comparison with covalent protein immobilization

	Ni-NTA Bead coupling procedure	Penta·His Bead coupling procedure	Covalent protein coupling procedure
Volume of beads required for 500 assay points	250 µl	250 µl	1 ml
Washing step	Not required	Not required	2 centrifugation steps
Activation step	Not required	Not required	EDC/Sulfo-NHS (1 h)
Protein required for 500 assay points	<5 µg	<5 µg	25–250 µg (optimization recommended)
Incubation	1 h or overnight	1 h or overnight	0.5 h – overnight
Additional steps required	Ready to use	Ready to use	Further wash step Enumeration of beads

Reference

1. *Zhang, J.H., Chung, T.D., and Oldenburg, K.R. (1999) A simple statistical parameter for use in evaluation and validation of high-throughput screening assays. J. Biomol. Screen.* ***4****, 67.*

Table 2. The Z-factor

The Z-factor is based on assay signal-to-noise and signal-to-background ratios.

$$\text{Z-factor} = 1 - \frac{3\sigma \text{ positive control} + 3\sigma \text{ negative control}}{\text{mean positive control} - \text{mean negative control}}$$

For the experiment presented in Figure 2 the following values were calculated.

Background (negative control): Mean = 18, σ (standard deviation) = 2.5
Positive control: Mean = 2524, σ (standard deviation) = 66.5

These data give a Z-factor for the assay shown in Figure 2 of 0.92.

Sensitive Multiplex Assays

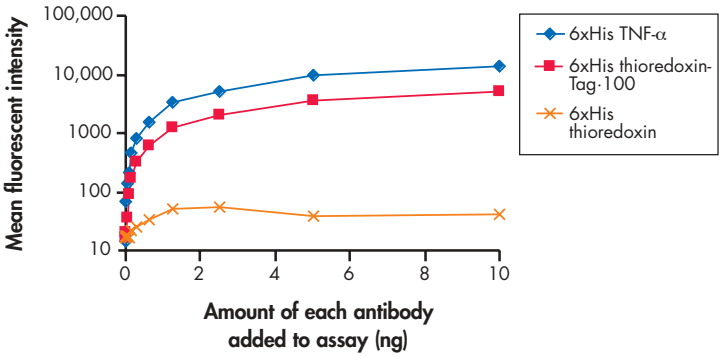

Figure 1 *Multiplex ELISA assay in which three different proteins were bound to LiquiChip Ni-NTA Beads using a common procedure, and used to quantify protein-specific antibodies.*

Highly Reproducible Assays

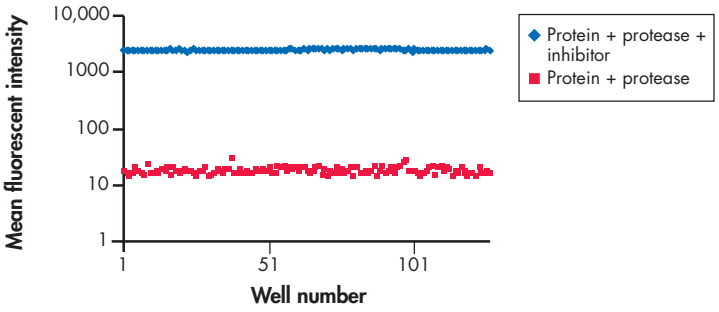

Figure 2 *High signal-to-background ratio and low background noise result in high Z-values. As a positive control, protease activity was inhibited with the serine-protease inhibitor aprotinin (10 ng/well). Data derived from 3 microtiter plates, processed on three successive days, are shown.*

By enabling fast assay development and setup and delivering high-quality data, the LiquiChip System is a valuable addition to any lab performing protein interaction assays. For more information on the LiquiChip System and how it can help you to streamline assay development visit **www.qiagen.com** or call QIAGEN Technical Services. ■