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Surface Plasmon Resonance imaging

How to quantify a protein in different crude samples in one run, using the XelPleX

Application Note
Biology
SPRi AN36

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Protein Y is a component of the HIV structure, it's commonly used as AIDS diagnosis tool, in combination with other immunological tests. Till now, ELISA is the standard method for Protein Y based diagnosis. Nevertheless, this method shows some limitations in terms of rapidity and high throughput which are more and more required in pharmaceutical research area.
SPRi technology is a good alternative to the use of ELISA in the protein concentration determination. Unlike ELISA which needs labelling and which is time consuming, SPRi is label free, and requires only few minutes to screen a huge panel of samples.
This application note shows how the protein Y can be detected and quantified in different supernatants with only a single injection using the XelPleX system and it proves the potential of the SPRi platform for high throughput concentration analysis in crude samples.

Materials and Method

Antibodies immobilization using SPRi-CFM on a SPRi-Biochip™ CH-LD

Figure 1 shows the assay set-up.

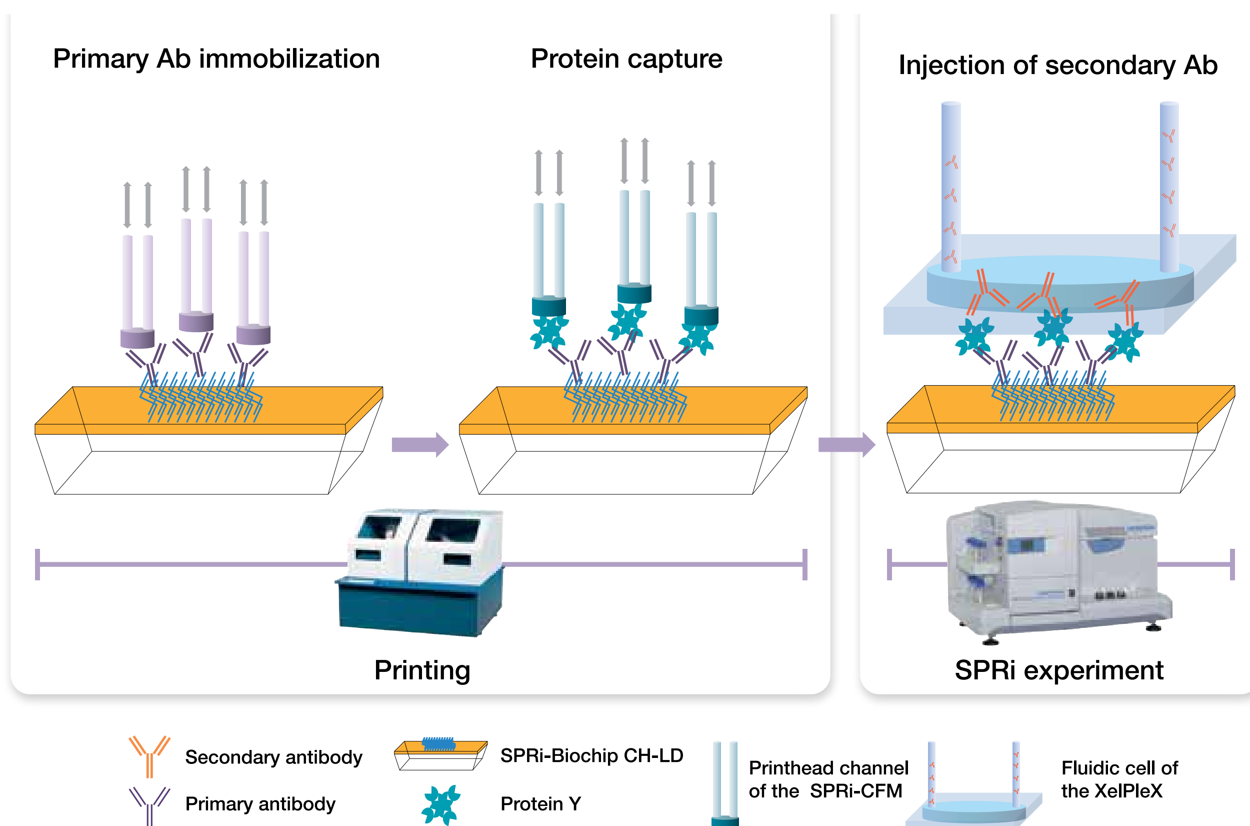


Figure 1: Assay set-up

Step 1: Primary antibodies immobilization

Primary antibodies directed against the protein Y were immobilized on a SPRi-Biochip™ CH-LD that is made of a self-assembled monolayer of polyoxyde ethylene glycol activated using an EDC/sulfo-NHS solution for amine coupling.

All the capture antibodies were prepared at 1 µM in 10 mM sodium acetate at pH 5.0 and immobilized on the activated surface of the SPRi-Biochip™ using the SPRi-CFM.
The SPRi-CFM uses flow deposition to immobilize up to 48 molecules in a single run (up to 144 spots per chip). Immobilizing with flow generates higher spot homogeneity and immobilization amount.

Step 2: Protein capture

The protein Y was captured at 10 ng/mL, 40 ng/mL, 120 ng/mL, 370 ng/mL, 1.1 µg/mL, 3.33 µg/mL and 10 µg/mL. The concentration range was chosen to cover the concentrations of the tested supernatants. The 5 supernatants tested were used without dilution.

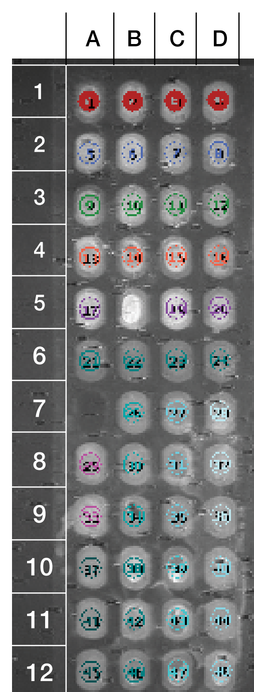
A negative control (anti-Ovalbumin IgG) and a positive control (protein Y spiked in crude medium) were also immobilized. Both purified protein Y and anti-Ovalbumin IgG were diluted in 10mM PBS pH7.4.

After the immobilization procedure, the SPRi-Biochip™ was blocked using 1M ethanolamine. Figure 2 shows the printing pattern and the image of the printed biochip.

SPRi experimental details

The printed SPRi-Biochip™ was then loaded into the XelPleX system, the interactions were monitored using EzSuite software. The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25°C. Then, 200 µL of the secondary antibody were injected into the fluidic system at a flow rate of 50 µL/min.

	A	B	C	D
1	Supernatant 1	Supernatant 1	Supernatant 1	Supernatant 1
2	Supernatant 2	Supernatant 2	Supernatant 2	Supernatant 2
3	Supernatant 3	Supernatant 3	Supernatant 3	Supernatant 3
4	Supernatant 4	Supernatant 4	Supernatant 4	Supernatant 4
5	Supernatant 5	Supernatant 5	Supernatant 5	Supernatant 5
6	NC	NC	NC	NC
7	PC	PY C5	PY C3	PY C1
8	PC	PY C5	PY C3	PY C1
9	PC	PY C5	PY C3	PY C1
10	PY C7	PY C6	PY C4	PY C2
11	PY C7	PY C6	PY C4	PY C2
12	PY C7	PY C6	PY C4	PY C2



NC	Negative control
PC	Positive control
PY C1	10 ng/mL

PY C2	40 ng/mL
PY C3	120 ng/mL
PY C4	370 ng/mL

PY C5	1.1 µg/mL
PY C6	3.3 µg/mL
PY C7	10 µg/mL

Figure 2: Printing pattern (left) and image of the SPRi-Biochip™ (right)
(Masks superimposed on the right image)

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Results and discussion

After a buffer injection, the secondary antibody was injected at 100 nM. Figure 3 shows the kinetic curves obtained for the purified protein Y captured at known increasing concentrations and for the supernatants (left) and on the protein Y captured from the five tested supernatants (zoom on the right).

As shown in Figure 3, the protein Y (P Y) protein was detected in all supernatants tested.
A calibration curve was drawn using the responses obtained for each concentration of purified protein Y immobilized (Figure 4). The curve was used to evaluate the concentration of the protein Y in the different supernatants in a single biochip and with a single injection.

Conclusion

The multiplex feature of the label-free interaction analysis platform from HORIBA Scientific enables protein quantification in several crude samples simultaneously, enabling to save time and money.
Through a single injection, we were able to determine the concentration of the protein Y in 5 different supernatants using a calibration curve established on the same SPRi-Biochip™.

This application demonstrates that the SPRi platform is a powerful tool for rapid and high throughput quantification applications.
On a single biochip, 14 different conditions were tested and 48 sensograms were generated in less than 15 min.

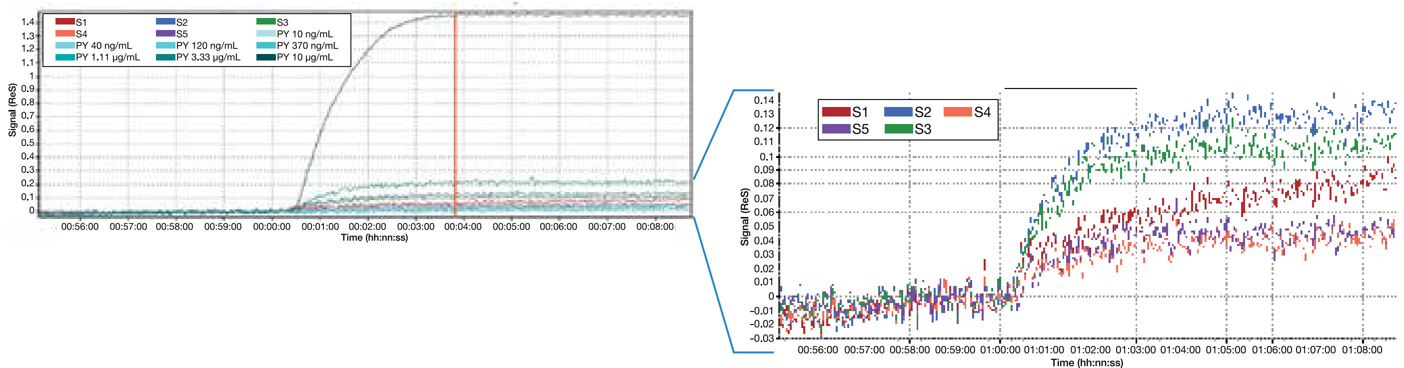
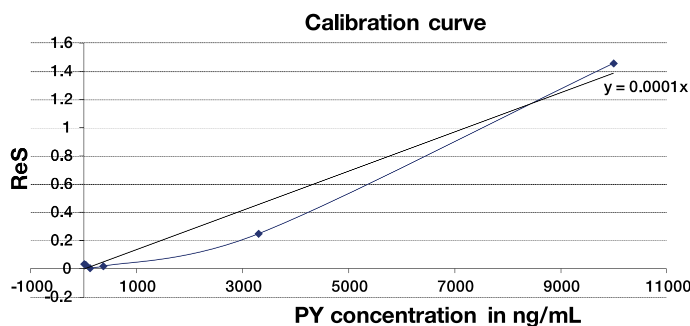


Figure 3: Averaged and reference-subtracted kinetics curves (left) and zoom on the curves corresponding to the 5 supernatants (right) after injection of the secondary antibody at 100 nM



Sample	P Y concentration in µg/mL
Supernatant 1	0.73
Supernatant 2	1.36
Supernatant 3	1.09
Supernatant 4	0.44
Supernatant 5	0.42

Figure 4: Calibration curve and protein Y concentrations determined in the supernatants

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