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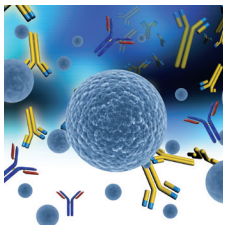
Surface Plasmon Resonance imaging

How can Multiplexing Speed up the Optimization of your SPRi Experiment? Example for an Antibody Biochip



Application Note
Biology
SPRi AN37

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The XelPleX instrument enables a large number of different label-free molecular interactions to be monitored simultaneously and in parallel in a single experiment. Throughput presents superior capacity in comparison with channel-based SPR systems, allowing fast matrix experiments and analyses to be undertaken.

In this technical note, the multiplexing capability of SPRi will be demonstrated for the optimization of antibody immobilization conditions on a SPRi-Biochip™. It will be focused on three different immobilization parameters. Firstly, the optimal ligands concentrations will be determined. Secondly, the performance of two commercially available anti-streptavidin antibodies will be compared. Thirdly, the goal is to find an ideal negative control antibody. The multiplex format of SPRi combines optimization of these three experimental conditions in a single experiment using a single biochip. Thus, parameters optimization can be easier, faster and cheaper in a multiplex configuration than in the conventional techniques such as ELISA.

Materials and Method

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

The SPRi-Biochip™ CH-LD is made of a self-assembled monolayer of polyoxyde ethylene glycol. A low density (LD) of reactive polyoxyde ethylene glycol is mixed together with non-reactive polyoxide ethylene glycol. The SPRi-Biochip™ CH-LD is activated using an EDC/sulfo-NHS solution in preparation for amine coupling.

The biochip contains several antibodies either useful for food (egg white) and pollen (birch) allergy detection or directed against proteins from biological fluids (serum, saliva).There are two antibodies anti-streptavidin from two different suppliers for comparison.

The different antibodies immobilized on the biochip are shown in Table 1.

Table 1: List of antibodies immobilized on the SPRi-Biochip™

Antibodies immobilized on the biochip	Supplier	Abbreviation
polyclonal anti-ovalbumin	TEBU	a-ova
polyclonal anti-streptavidin	TEBU	a-strepta SIGMA
polyclonal anti-streptavidin	SIGMA	a-strepta TEBU
polyclonal anti-lectin	SIGMA	a-lect
polyclonal anti-transferrin	TEBU	a-trans
polyclonal anti-α amylase	SIGMA	a-amy
monoclonal anti-Bet v 1	STALLERGENES	a-Bet v1
polyclonal mouse IgG (negative control)	SIGMA	mIgG

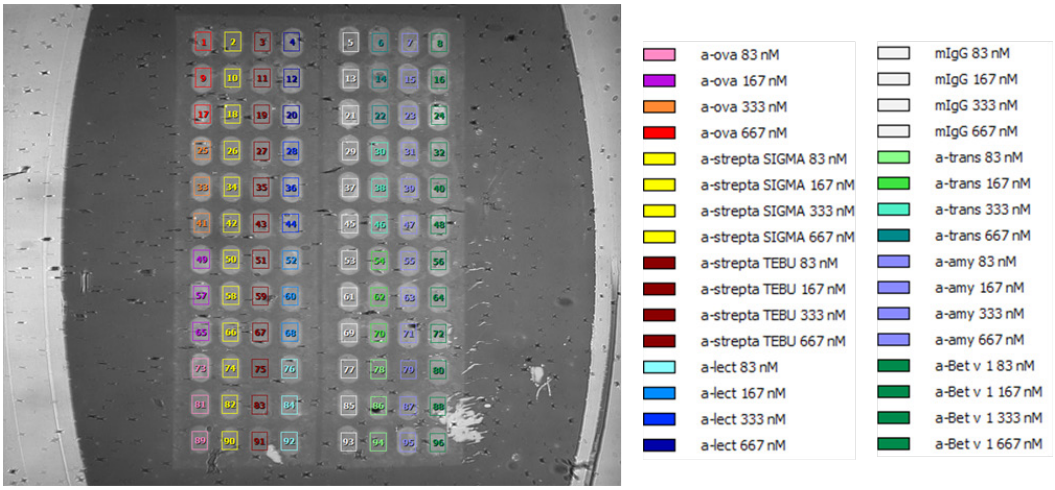

Figure 1: Image of the printed SPRi-Biochip™ and spotting matrix

Figure 1 shows the pattern used for immobilizing molecules in an array format (spots). All antibodies were prepared in 10 mM sodium acetate at pH 4.5 at four different concentrations using a two-fold dilution series: 83 nM, 167 nM, 333 nM and 667 nM, and immobilized to 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 printing run. Three printing runs can be performed on a single biochip (up to 144 spots per chip). The microfluidic immobilizing generates higher spot homogeneity and immobilization level.
Each antibody in each condition was immobilized in triplicate. Two printing runs of the SPRi-CFM were performed leading to a total of 96 spots.

After the immobilization procedure, the SPRi-Biochip™ was blocked using 1 M ethanolamine and saturated using 1 % BSA.

SPRi experimental details

The printed SPRi-Biochip™ was then loaded into the XelPleX™ system. The running buffer was 10 mM PBS pH 7.4 and the working temperature was set to 25°C.
Then, 200 µL of each corresponding antigen were injected into the fluidic system at a flow rate of 50 µL/min. The surface was regenerated using 0.1 M glycine-HCl pH 2.0 or 10mM NaOH after each antigen injection.

Results and Discussion

Optimization of antibodies immobilization concentration

To illustrate this part, two different examples of results will be discussed using anti-ovalbumin, anti-lectin and anti-transferrin antibodies.
Figures 2A-3A show the averaged binding kinetics obtained respectively on the anti-ovalbumin spots and on the anti-lectin spots after the subtraction of the negative control (mIgG).

The highest response level is obtained when the antigens (ovalbumin and lectin) interact with their antibody immobilized at 667 nM (the highest concentration tested) on the SPRi-Biochip surface. Immobilizing the antibody at lower concentrations yielded a lower response. One should test immobilizing the anti-ovalbumin antibody and the anti-lectin antibody at higher concentrations than 667 nM to define the saturation level.

The anti-ovalbumin antibody is also highly specific as the signal from the other antibodies is negligible as observed by the kinetic curves (Figure 2A) and the SPR difference image (Figure 2B). White spots on the SPR difference image correspond to areas where binding has occurred.
On the contrary, the anti-lectin antibody is weakly specific as low signals are observed from the other antibodies as observed by the kinetic curves (Figure 3A) and the SPR difference image (Figure 3B). White spots on the SPR difference image correspond to areas where binding has occurred.

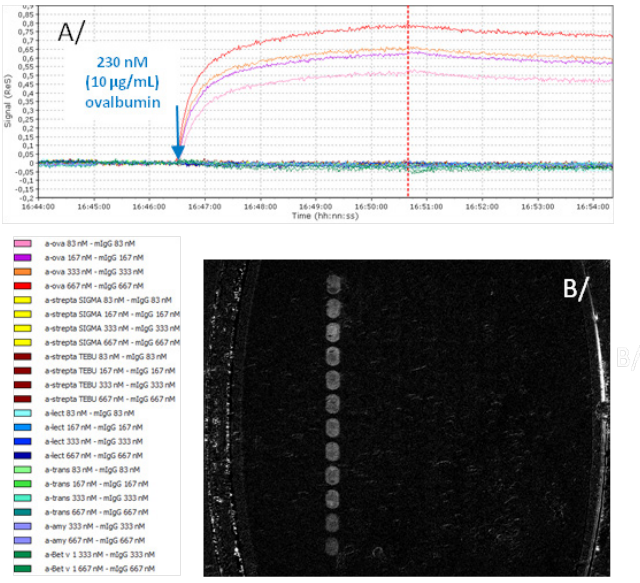

Figure 2: (A) Averaged kinetic curves (3 spots) after subtraction of the negative control (mIgG) obtained for the injection of 230 nM (10 µg/mL) ovalbumin. (B) Difference image obtained for the injection of 230 nM (10 µg/mL) ovalbumin.

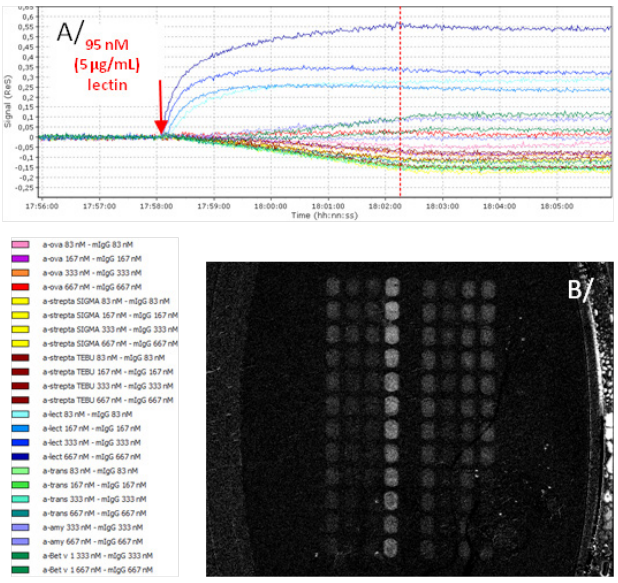

Figure 3: (A) Averaged kinetic curves (3 spots) after subtraction of the negative control (mIgG) obtained for the injection of 95 nM (5 µg/mL) lectin. (B) Difference image obtained for the injection of 95 nM (5 µg/mL) lectin.

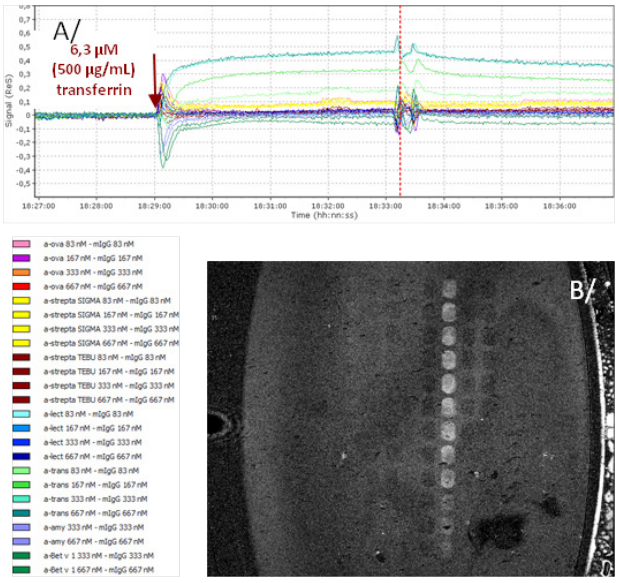

Figure 4: (A) Averaged kinetic curves (3 spots) after subtraction of the negative control (mIgG) obtained for the injection of 6.3 µM (500 µg/mL) transferrin. (B) Difference image obtained for the injection of 6.3 µM (500 µg/mL) transferrin.

Figure 4A shows the averaged binding kinetics obtained on the anti-transferrin spots after the subtraction of the negative control (mIgG). Highest response levels are obtained when the transferrin interacts with its antibody immobilized both at 333 and 667 nM on the SPRi-Biochip surface. Saturation level of the anti-transferrin antibody is reached and is close to the concentration of 333nM. Immobilizing the antibody at lower concentrations yielded a lower response.
On the SPR difference image obtained during injection of transferrin (Figure 4B), the whole surface is slightly coloured in white revealing that the transferrin solution injected has a different refractive index than the one of the running buffer. A bulk increase is therefore induced on the kinetic curves without negative control subtraction attributing to the peaks observed at the beginning and at the end of the transferrin injection on the reference-subtracted curves (Figure 4A).

On the SPR difference image obtained during injection of transferrin (Figure 4B), the whole surface is slightly coloured in white revealing that the transferrin solution injected has a different refractive index than the one of the running buffer.

A bulk increase is therefore induced on the kinetic curves without negative control subtraction attributing to the peaks observed at the beginning and at the end of the transferrin injection on the reference-subtracted curves (Figure 4A).

This study was performed for all the antibodies immobilized on the SPRi-Biochip™. The synthesized results are shown in Figure 5.
Bar chart of Figure 5 compares specific responses obtained for the different antibodies immobilized at four different concentrations after injection of their respective antigen. The optimal concentrations determined for each antibody are circled in Figure 5 and are summarized in Table 2.
Looking at these optimal concentrations determined, a tendency can be stressed. We noticed that the optimal immobilization concentration is lower for the big molecules such as streptavidin and higher for the small molecules like Bet v 1. This can be explained by a steric hindrance issue.

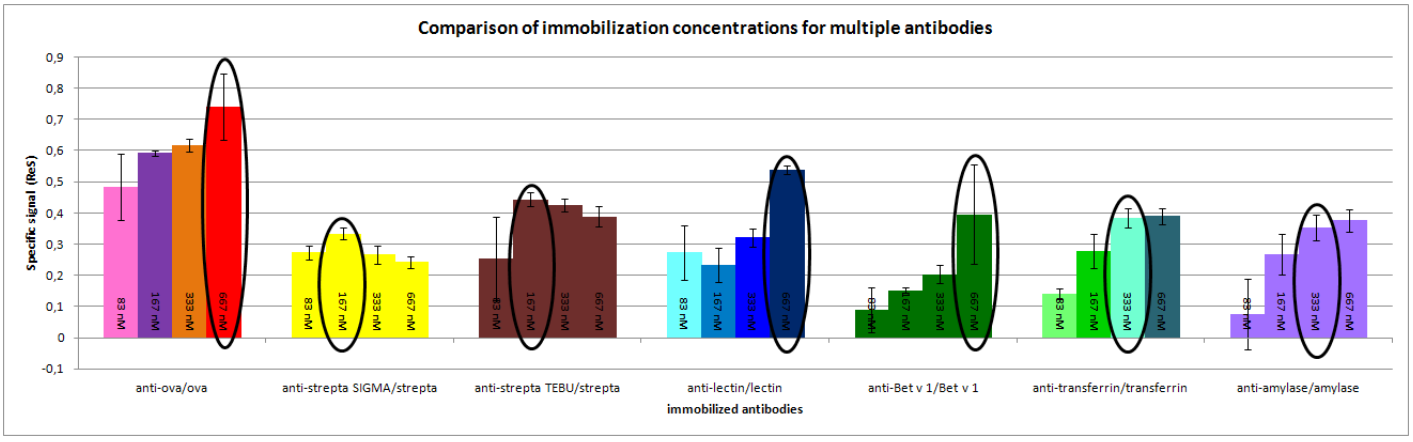

Figure 5: Specific responses obtained for the different antibodies immobilized at four different concentrations

Table 2: Optimal immobilization concentration determined for the different antibodies studied

Model studied		Molecular mass (Da)	Optimal immobilization concentration (nM)
Antibody	Antigen		
polyclonal anti-ovalbumin	ovalbumin	44300	667
polyclonal anti-streptavidin SIGMA	streptavidin	60000	167
polyclonal anti-streptavidin TEBU	streptavidin	60000	167
polyclonal anti-lectin	lectin	53000	667
polyclonal anti-transferrin	transferrin	80000	333
polyclonal anti-α amylase	α amylase	62000	333
monoclonal anti-Bet v 1	Bet v 1	17000	667

Comparison of different antibodies providers

Working in an array format allows for the rapid comparison of different antibodies targeting the same antigen. To illustrate the antibodies comparison, here, two antibodies targeting streptavidin (from two different providers) are immobilized on the same biochip at the same concentrations. Only one injection of the antigen permits to compare each antibody response. The anti-streptavidin antibody from TEBU gives the highest SPR response (about 20 % more signal) as observed on Figure 6A. The two anti-streptavidin antibodies are also highly specific as the signal for the other antibodies is negligible on the kinetic curves (Figure 6A) and on the SPR difference image (Figure 6B). White spots on the SPR difference image correspond to areas where binding has occurred.

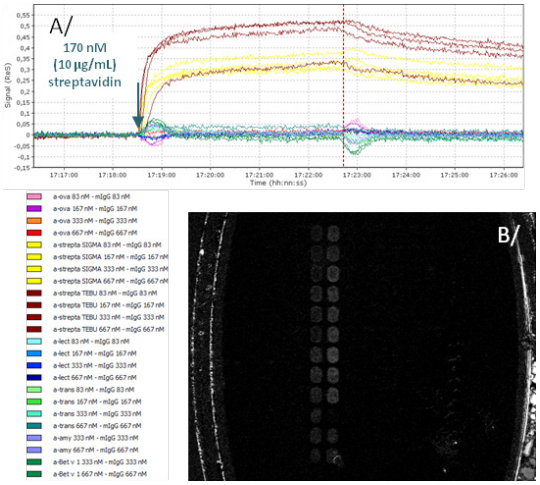

Figure 6: (A) Averaged kinetic curves (3 spots) after subtraction of the negative control (mIgG) obtained for the injection of 170 nM (10 µg/mL) streptavidin. (B) Difference image obtained for the injection of 170 nM (10 µg/mL) streptavidin.

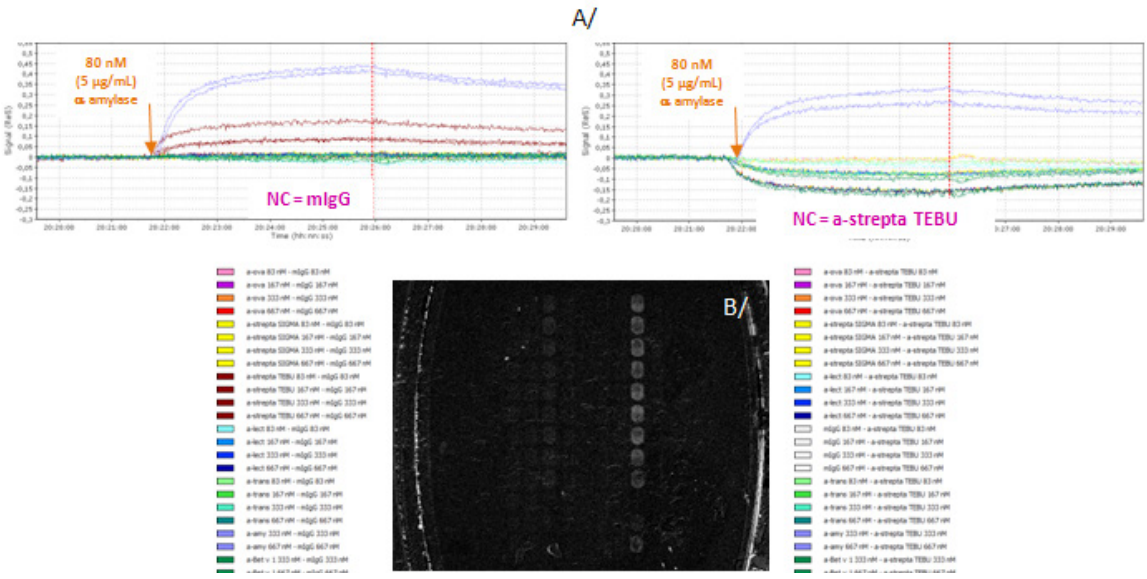

Figure 7: (A) Averaged kinetic curves (3 spots) obtained for the injection of 80 nM (5 µg/mL) α amylase. Subtraction using mIgG as negative control (left) compared to subtraction using anti-streptavidin TEBU as negative control (right). (B) Difference image obtained for the injection of 80 nM (5 µg/mL) α amylase.

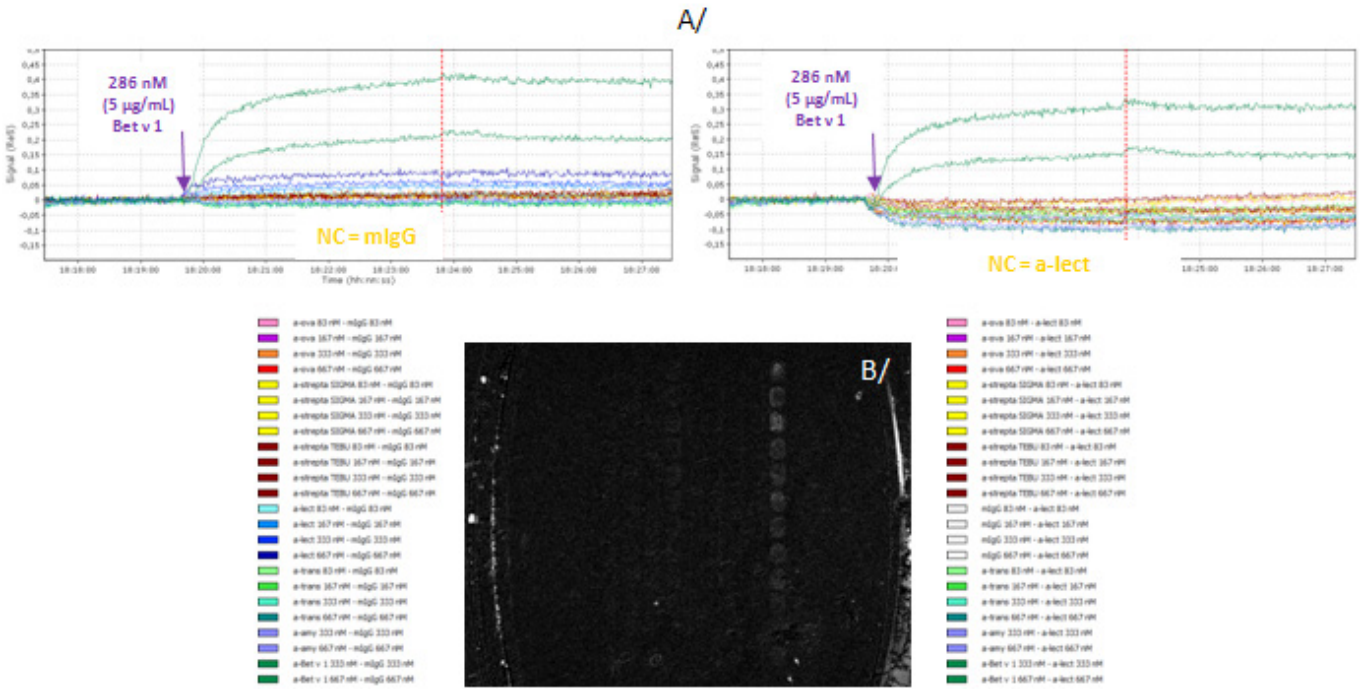

Figure 8: (A) Averaged kinetic curves (3 spots) obtained for the injection of 57 nM (5 µg/mL) Bet v 1. Subtraction using mIgG as negative control (left) compared to subtraction using anti-lectin as negative control (right). (B) Difference image obtained for the injection of 57 nM (5 µg/mL) Bet v 1.

The choice of the appropriate negative control can be a compromise

The specificity of the antigen-antibody interaction can also be easily determined using the multiplex approach. For example, on left graphs of Figures 7A-8A and on the SPR difference images (Figures 7B-8B), some non specific binding of α amylase can be observed on anti-streptavidin antibody from TEBU supplier after injection of α amylase at a concentration of 80 nM and some non-specific binding of Bet v 1 can be observed on anti-lectin antibody after injection of Bet v 1 at a concentration of 57 nM, respectively.

To determine the accurate specific binding responses of anti-α amylase and anti-Bet v 1 antibodies, anti-streptavidin antibody TEBU and anti-lectin antibody respectively can be used as negative controls and subtracted results can be compared (right graphs of Figure 7A and Figure 8A). Specific binding responses are around 25 % lower using anti-streptavidin antibody TEBU and anti-lectin antibody as negative controls compared to mouse IgG antibody. Nevertheless, some negative signals are observed on some kinetic curves due to the non-specific responses obtained by anti-streptavidin TEBU and anti-lectin spots.

Therefore, the choice of the negative control can be adapted to the aim of the application.

Conclusion

A key feature of the Surface Plasmon Resonance imaging technology is the ability to multiplex.

This technical note illustrates how the multiplex configuration can be useful for optimizing ligands immobilization conditions and consequently editing procedures for some specific biochip preparations dedicated to some applications (allergy, serology, pharmacology...). Indeed, working in an array format allows the direct comparison of the binding events occurring on the biochip in a single experiment. The results can be summarized on the Table 3. As a perfect example here, a total of twenty-eight different experimental conditions were tested and 576 different sensorgrams were generated in a single experiment using a single biochip, saving on time and consumable costs.

Table 3: Summary of the best conditions determined

Antibodies	Best concentration (nM)	Best provider
polyclonal anti-ovalbumin	667	
polyclonal anti-streptavidin	167	TEBU
polyclonal anti-lectin	667	
polyclonal anti-transferrin	333	
polyclonal anti-α amylase	333	
monoclonal anti-Bet v 1	667	

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