LE-SPR Validation Kit

The Label-Enhanced SPR Validation Kit B9003 consists of one coloured and one uncoloured sample of known compositions and optical properties. The kit can be used to learn how to set up, perform, and evaluate a LE-SPR experiment, or used as a positive control sample during regular LE-SPR analysis. Alternatively, the kit can be used to perform rigorous injection quality testing of a SPR instrument. This Tech Note describes the preparation and use of the kit.

Contents

1. Introduction

The LE-SPR Validation Kit consists of three components:

- A pre-weighed amount of Episentec™ dye.
- A pre-weighed amount of salt.
- Buffer tablets for buffer preparation.

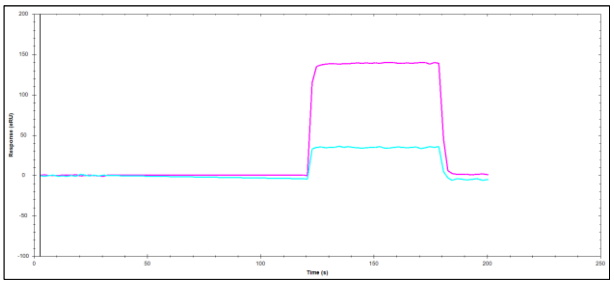
The dye and the salt are dissolved in buffer, yielding samples of known composition and optical properties. The dye yields a coloured solution with a very high refractive index. The salt yields a colourless solution with an increased refractive index.

The samples are injected and analysed on a SPR instrument, e.g. a Biacore™ instrument, in LE-SPR mode. The data is evaluated using EpiGrammer™ software to give enhanced sensorgrams (or 'epigrams') of known appearance. The samples will only yield square-shaped bulk signals in SPR analysis and are not expected to show any binding to the sensor surface. The dye and the salt are selected to show practically zero non-specific binding to common sensor surfaces.

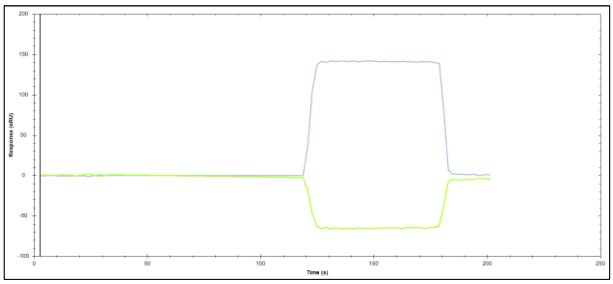
From a system perspective – optics as well as microfluidics - the kit test procedure is very simple and straightforward, and consequently the kit is well suited as a learning tool or test tool for LE-SPR. The high accuracy of the optical readout of LE-SPR further allows stringent diagnostics of injection pulse quality, providing information beyond that of standard SPR system check procedures.

2. Basic application: Instructions for use

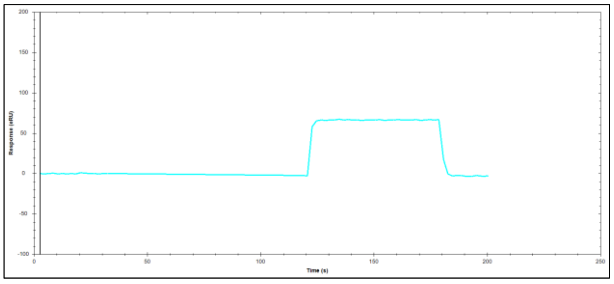
1. Dissolve the two buffer tablets carefully in 200 ml water.
2. Use 5 ml of the buffer to carefully dissolve the dye sample. The most practical way to do this may be to fill the sample vial with buffer and then transfer the formed solution to a larger vial. Repeat this several times and allow enough time to ensure complete dissolution and transfer of the dye. Avoid unnecessary evaporation during this procedure.
3. Use 5 ml of the buffer to carefully dissolve the salt sample in the same way.
4. It is recommended to filter and degas all liquids according to standard SPR practices.
5. Use the prepared buffer as the running buffer on the SPR instrument.
6. Use a well-defined and stable sensor chip, e.g. CM-dextran. Avoid chips with proteins.
7. Run a LE-SPR experiment with injection of the dye sample and the salt sample. Start by running at least five buffer injections to equilibrate the instrument. Run the samples at least in triplicates. Detailed instructions on how to set up and run a LE-SPR experiment are found in additional Technical Notes:
    - For Biacore™ 2000/3000: Technical Note 004.
    - For Biacore™ T100/T200: Technical Note 005.
8. Export the data to EpiGrammer™ for analysis and enhancement. Detailed instructions on how to export data are found in additional Technical Notes:
    - For Biacore™ 2000/3000: Technical Note 002.
    - For Biacore™ T100/T200: Technical Note 003.
9. Analyse the data in EpiGrammer according to Technical Note 009, which is a tutorial on data evaluation that utilizes Validation Kit data as a calculation example. The expected outcome of the data analysis of the dye sample is a square-shaped epigram. The salt sample should yield an epigram with practically zero signal.



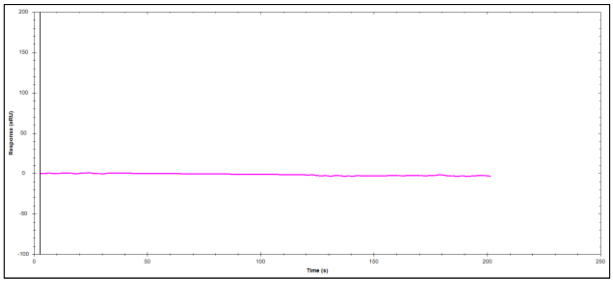
Dip position DP (red) and dip width DW (blue) of dye sample.



Dip position DP (blue) and dip width DW (green) of salt sample.



Epigram of dye sample.



Epigram of salt sample.

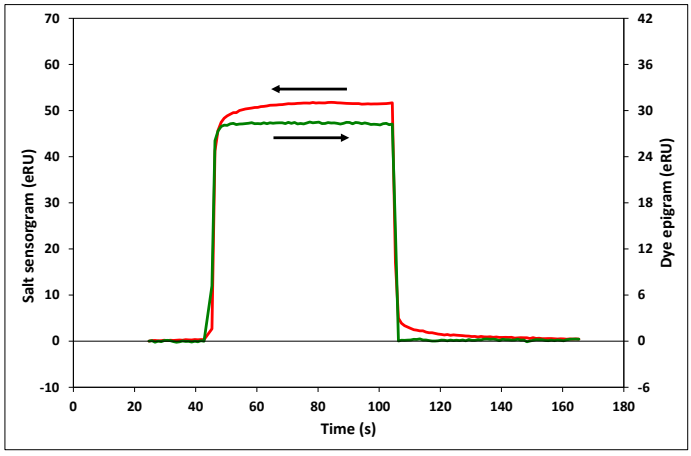
3. Advanced application: Using the Validation Kit for injection pulse quality diagnostics

Most commercial SPR instrument systems, e.g. Biacore™ systems, offer pre-programmed "System Check" procedures for assessing the instrument performance. These procedures usually include checking the injection pulse quality in order to detect e.g. leakage, blockage, or dispersion in the microfluidic system. Standard injection tests are based on injection of a solution with a different refractive index (RI) than that of the buffer – in parallel with the salt solution of the LE-SPR Validation Kit. However, tests based on pure RI measurements are plagued by a number of well-known errors and artefacts [1-4]:

- Temperature variations.
- Pressure variations.
- Impurities in the flow system; detergent concentration variations; memory effects; spurious adsorption or desorption of molecules to or from the surface.
- Matrix effects; compression or expansion of the dextran matrix due to variations in pH or ionic strength.
- Carry-over or mixing of samples between injections.
- Other undefined artefacts: the overall drift specification of Biacore T200 and Biacore 3000 is 0.3 RU/min, amounting to 1 RU for a 3-minute cycle.

To allow for such RI errors, the tolerance limits of standard System Checks must be set unnecessarily wide, and consequently actual problems with the injection pulse quality may not be revealed. LE-SPR offers a more rigorous injection test, which monitors only the true transport of analyte in to and out of the detection flow cell with any RI artefacts excluded.

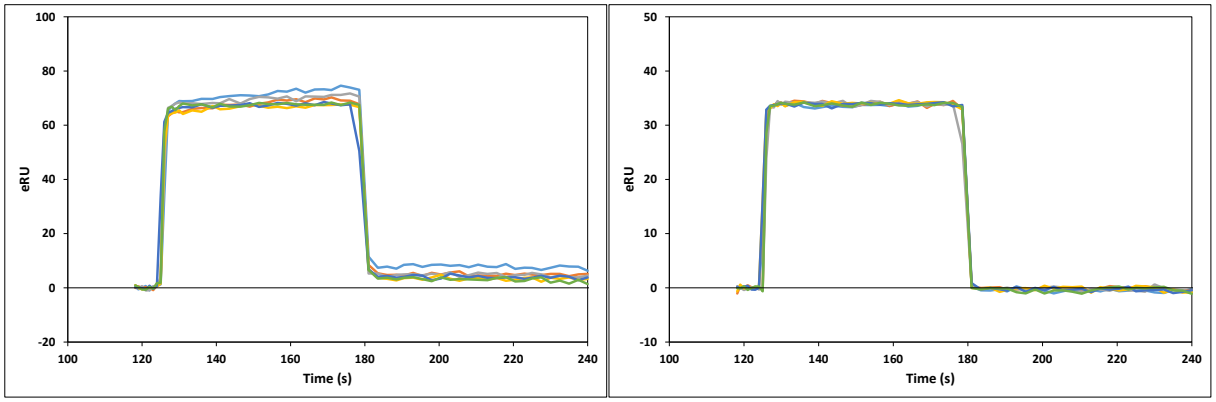
A first comparison of Validation Kit salt and dye injections on a Biacore™ T200 is shown in the graph below. The standard DP sensorgram of the salt injection requires about 30 seconds to reach plateau level. After the injection there is a remaining 4 eRU residual signal, and it takes more than 30 seconds to again reach baseline level. The epigram of the corresponding dye injection, on the other hand, reaches plateau level within the time constant of the instrument (a few seconds) and returns immediately to baseline level after the injection. Obviously, LE-SPR shows that the injection pulse as such is flawless, while undefined artefacts make the pure RI diagnostic procedure unreliable.



Biacore T200: Salt injection sensorgram (red) and dye injection epigram (green) scaled to visually same height.

A second comparison on a Biacore™ 2000 is shown in the graphs below. Six replicates of alternating salt and dye injections are depicted. The salt sensorgrams show a 3-5 eRU linear drift on top of the pulse, and there is an irreversible 4-8 eRU baseline shift after the pulse. The repeatability is poor. In clear contrast, the interspersed dye epigrams are free from drift and

baseline shifts, and the repeatability is excellent. Again, the performance of the injection system is perfect, even though pure refractive index measurements fail to demonstrate this fact.



Biacore 2000: Six salt injection sensorgrams (left) and six dye injection epigrams (right) scaled to visually same height.

Conclusion: LE-SPR can be used to assess the true shape of injection pulses on SPR instruments.

Notes

1. The Validation Kit should be used for qualitative purposes only, and not for accurate calibration. The expected maximum signal will depend on e.g. the SPR instrument used and the sensor surface used. Also, due to the inherent variations in the manufacturing of small kits, there may be a slight variation between different kits. However, for repeated measurements of one kit on the same instrument and the same sensor surface, the optical precision is high. The actual between-injection precision will generally reflect the injection repeatability and carry-over characteristics of the instrument.
2. The dye and salt samples may be dissolved in other buffers, e.g. when the kit is used as a positive control sample during regular LE-SPR analysis. Avoid pH values above 8, and make sure that the dye is completely dissolved in the buffer. Be sure to use the running buffer to dissolve the samples. Please note that the expected maximum signal will vary depending on the buffer used.
3. Validation Kit samples in solution should be stored in the dark (wrap in aluminium foil) in a refrigerator. Samples should be used within a few days.

References

1. R. Karlsson, "Experimental Design", Chap. 2 in M.A. Cooper (ed.), "Label-Free Biosensors, Techniques and Applications", Cambridge University Press, Cambridge, 2009.
2. R. Karlsson, A. Fält, "Experimental design for kinetic analysis of protein-protein interactions with surface plasmon resonance biosensors", J. Immunol. Methods, 200 (1997) 121.
3. Biacore 3000 Instrument Handbook, GE Healthcare, BR-1003-81 AG 07/2008, 2008.
4. Biacore T200 Data File, GE Healthcare, 28-9794-15 AA 06/2010, 2010.

Episentum AB
Karins väg 5, 194 61 Upplands Väsby, Sweden
Phone: +46 (0)72 007 79 08
E-mail: sales@episentum.com
www.episentum.com