SARTORIUS

Best Practice Guide

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Best Practice Guide: Octet® SPR Sensor Chip Preparation

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Introduction

Octet® SPR sensor chips allow you to generate high-quality surface plasmon resonance (SPR) data and this requires that a stable baseline is achieved prior to performing assays. An important parameter contributing to a stable baseline is a stable sensor chip surface. Best practice for preparing Octet® SPR sensor chips includes four basic steps that should be performed sequentially prior to starting assays:

- Normalization
- Priming
- Conditioning
- Hydration

Octet® SPR sensor chips are supplied in a sealed packet containing an inert gas and should be stored at the recommended condition upon delivery. Prior to installation, move the sensor chip to room temperature and allow to equilibrate without opening the packet (~10 mins). Once temperature equilibrated the sensor chip is ready for installation.

Normalization

Normalization is an important step in sensor chip preparation as individual sensor chips contain slight differences in their surface properties. Therefore, it is important to adjust the system's detector to compensate for these differences.

Normalization of the sensor chip should generally be performed after the following events:

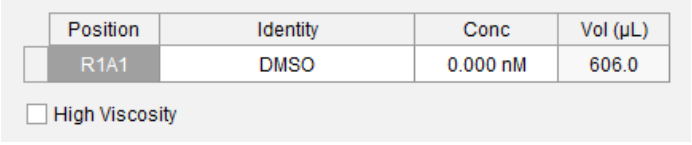
- Installation of a new sensor chip for the first time
- Installation of a sensor chip that has been previously installed
- When the assay temperature has been changed

The Octet® SF3 allows rapid normalization of the sensor chip using 100% DMSO and is located under **Instrument Setup: Normalize.**

Note: 100% DMSO: is typically used for normalization on a new sensor chip which has not been previously used or had a molecule immobilized on its surface. 70% glycerol is typically used when normalization of sensor chip with an immobilized ligand is required, this includes sensor chips that contain pre-immobilized ligands such as SADH.

Place the required solution in a sample rack as shown below and drag across to the empty editor box:

Item	Description	Position	Volume (µL)
Normalize	100% DMSO	R1A1	700

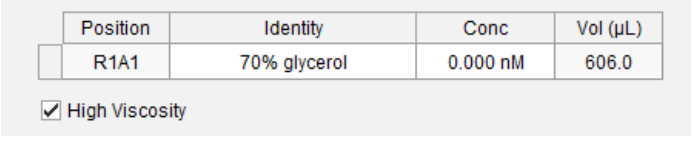


70% Glycerol Normalization

When performing normalization on a sensor chip that has material immobilized on it, 70% glycerol is recommended.

Item	Description	Position	Volume (µL)
Normalize	70% glycerol	R1A1	700

It is important that the High Viscosity box is ticked when using 70% glycerol.



Priming

Priming is an important part of sensor chip installation as it introduces running buffer to the flow cells of the sensor chip and aids in rehydrating the sensor chip surface after storage.

The prime function is located under **Instrument Setup: Prime.**

Best practice for priming is to perform a single prime when a new sensor chip is installed and normalized, and the conditioning steps shown below used. Three primes should be performed when the running buffer is changed to ensure the Octet® SF3 system and sensor chip are primed into the new buffer.

Conditioning

Prior to immobilization of a ligand onto the sensor chip it is recommended that the sensor chip is initially cleaned and hydrated to ensure the best results. Therefore, after priming it is recommended that sensor chips are preconditioned using the Fast injection type option with the following conditioning solutions prior to immobilization:

COOH1, CDL, CDH and PCH

Injection	Solution	Contact time (sec)	Flow rate (µL min^{-1})
1	10 mM HCl	15	100
2	50mM NaOH	15	100
3	0.1% (w/v) SDS	15	100
4	10 mM HCl	15	100
5	50mM NaOH	15	100
6	0.1% (w/v) SDS	15	100

SADH

Injection	Solution	Contact time (sec)	Flow rate (µL min^{-1})
1	1 M NaCl 50mM NaOH	15	100
2	1 M NaCl 50mM NaOH	15	100
3	1 M NaCl 50mM NaOH	15	100
4	1 M NaCl 50mM NaOH	15	100

HisCap

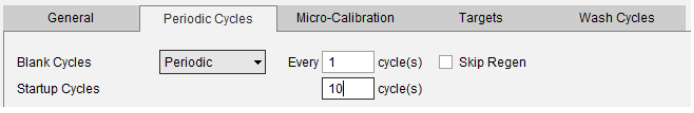
Injection	Solution	Contact time (sec)	Flow rate (µL min^{-1})
1	400 mM EDTA	15	100
2	400 mM EDTA	15	100
3	400 mM EDTA	15	100
4	400 mM EDTA	15	100

Following conditioning, it is best practice to perform a single prime with the running buffer to help ensure a stable baseline prior to performing further surface preparation.

Hydration and Post-Conditioning

After immobilization, it is vital to ensure that temperature fluctuations and more commonly, swelling of the sensor chip matrix does not occur. This ensures that a stable baseline is achieved prior to performing an SPR assay, which leads to higher quality data. With the Octet® SPR Discovery software this is easily achieved by including startup cycles in methods.

Located in the **Periodic Cycles** section of the **Method Editor**; startup cycles are performed at the start of the assay and are not included in subsequent blank cycles.



It is recommended that 5 – 10 startup cycles are included in the method to help ensure a stable baseline prior to performing the assay.

It is recommended that post-conditioning of the sensor chip is also performed prior to collecting data in order to remove any material that is not covalently attached to the sensor chip surface and to expose the sensor chip matrix to potential pH shock prior to collecting data.

Post-conditioning is achieved by performing ~5 cycles of analyte binding (Association) and regeneration using the same parameters as in the main assay. Post-conditioning uses the lowest concentration of analyte and the same regeneration solution to be assessed in the assay.

For example, in a multi-cycle kinetics assay with the following parameters:

	Association (Sec)	Dissociation (Sec)	Flow rate (µL min^{-1})
Analyte 0.411 – 100 nM	180	600	50
Regeneration 100 mM HCl	30	NA	50

Post-conditioning would consist of ~5 cycles:

	Association (Sec)	Dissociation (Sec)	Flow rate (µL min^{-1})
Analyte 0.41 nM	180	600	50
Regeneration 100 mM HCl	30	NA	50

Where OneStep® injections are performed, a 200-fold dilution of the single concentration of analyte should be used.

Conclusion

With proper storage and preparation, Octet® SPR sensor chips can be used to generate real-time kinetics and affinity data for a wide range of molecules.

When combined with the best practices of Normalization, Priming, Conditioning and Hydration, which help ensure a stable sensor chip and baseline prior to performing your method, Octet® SPR Sensor Chips and the Octet® SF3 can generate high quality data for even the most challenging assays.

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