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Creoptix Protein Immobilization Protocol: Biotin Capture on Streptavidin Chip

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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Abstract

This protocol provides a quick yet comprehensive method for the capture of various biotinylated proteins on a PCH-STA chip, that preserves protein activity. The procedure covers system preparation, chip conditioning, protein capture, and subsequent analysis using the Creoptix WAVEsystem. It is a methods of surface modification that includes surface priming with the running buffer, protein capture, and final wash steps. The protein capture step requires optimization of the target protein concentration and injection duration to achieve desired response levels.

Materials

PCH-STA:
PCH-STA Pre-immobilized streptavidin, thick PC hydrogel **Malvern Panalytical Catalog #**9060010
Borate buffer: 0.1 M sodium borate, 1 M NaCl pH 9.0 **XanTec bioanalytics GmbH Catalog #**B BELU-50ML
10x HBS-P: HBS-P Buffer 10× **Cytiva Catalog #**BR100671

Safety warnings

Do not set the Flow Cell Temperature below 10 and/or 15°C. At lower temperatures, condensation occurs on the chip.

Before start

The composition of the running buffer used will differ for each protein. To ensure the best chances of success, conduct a thorough literature search for information on you target protein’s buffer stability and any relevant experimental parameters from published immobilization assays.

Instructions:

1 The running buffer will differ for each protein. We highly recommend that a literature review should proceed any study to gather background information on protein stability and relevant experimental parameters from other immobilization assay used previously.

2 Variables that are for the immobilization steps indicated the table below.

	A	B
	Running buffer composition	**[X]**
	Protein concentration (µg/mL)	**[Y]**
	Protein injection duration (s)	**[Z]**

Variables definitions

Setting up WAVEsystem 20m

3 Prepare 200 mL **running buffer** by diluting stock solution (normally 10x) with Miili-Q water. 20m

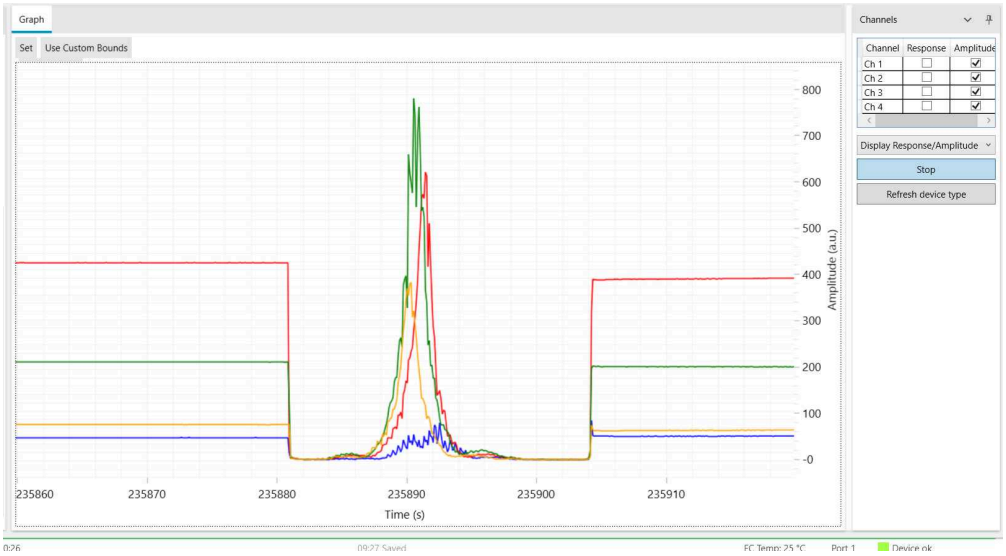
3.1 Degas the buffer for 00:02:00 in an ultrasonic bath. 2m

3.2 Keep 40 mL **running buffer** aside for preparation of samples and other solutions.

4 Insert the tubes into the remainder of the **running buffer** and insert the PCH-STA chip into the instrument.

4.1 Select "***new buffer and new chip"*** in the pop-up window. This will prime the system and perform the chip alignment.

4.2 During the chip alignment, check the profile and the amplitude levels at the end of the aligment step.



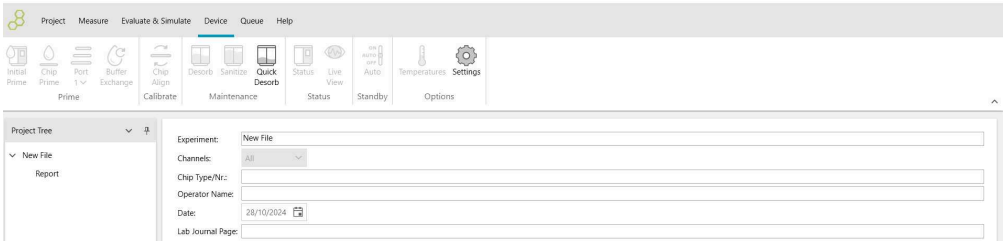
Example amplitude profile during the chip alignment.

Note

Manual checks to determine if the chip is functioning properly:
1) The amplitudes for all channels should be above 50 (a.u.)
2) During chip alignment the all amplitude profiles shows triangular spikes which return to flat baselines at the end of the alignment

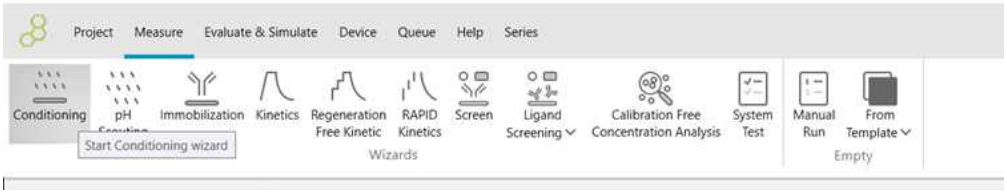
PCH-STA chip conditioning

5 In the **Device** tab, start **Priming** and **Chip Prime.** Repeat at least 2x.



Screenshot of Creoptix software to perform Prime and Chip prime

6 To condition the chip go to **Conditioning** on the **Measure** tab.

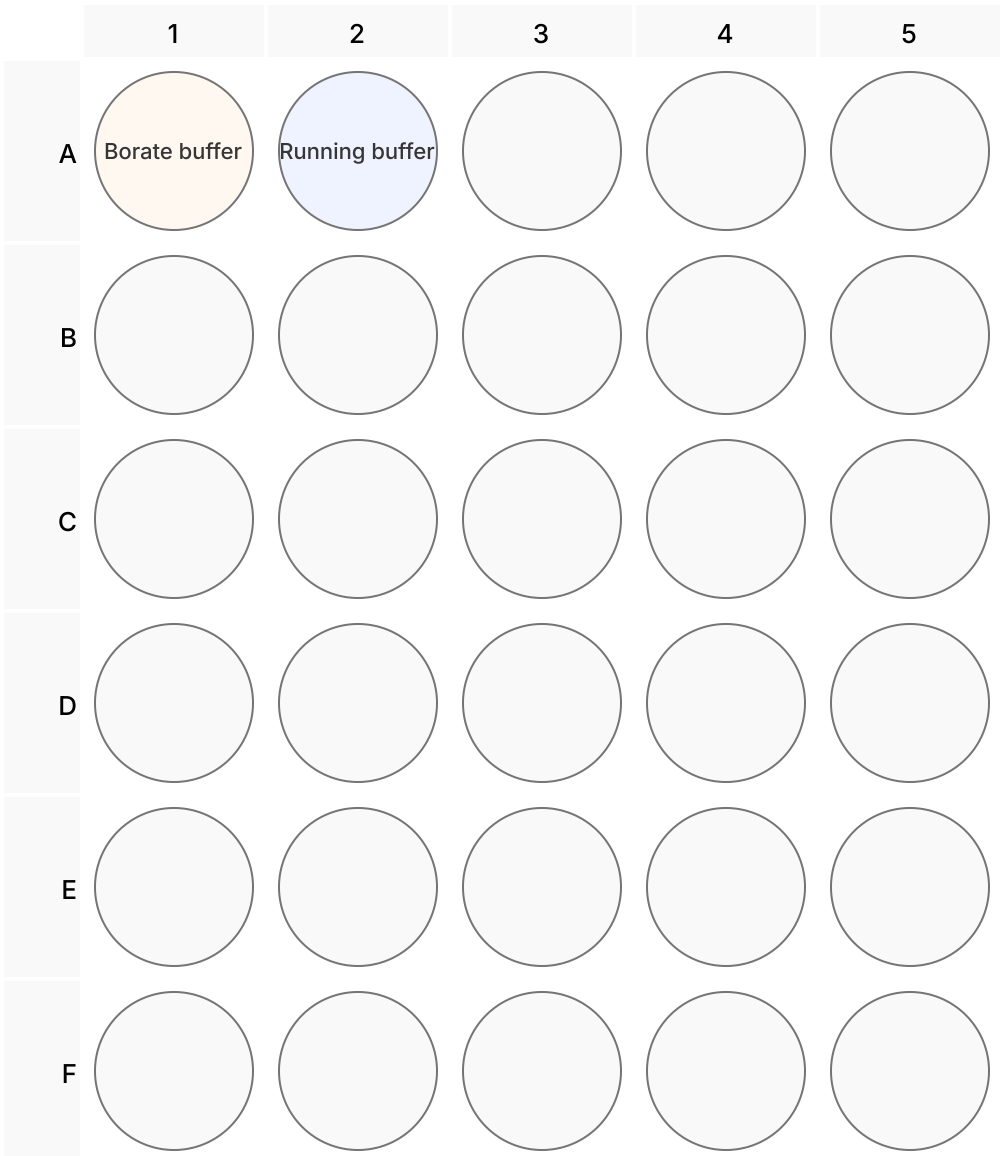


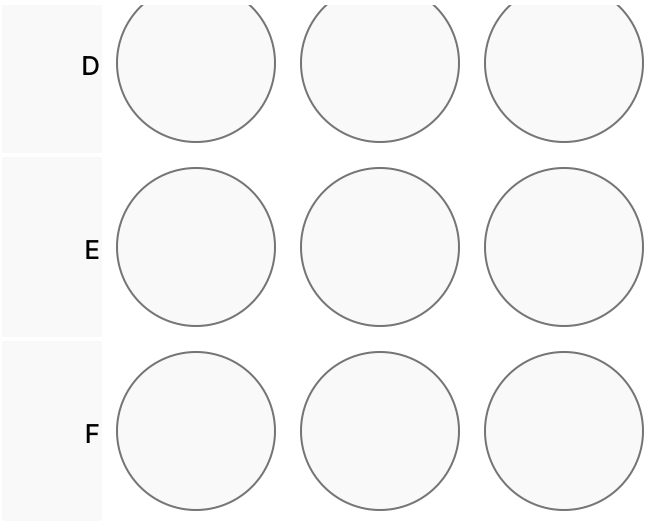
Screenshot of Creoptix software to perform Conditioning

6.1 Select chip type as **PCH-STA** chip.

6.2 Define the running buffer.

6.3 Create series and prepare samples as indicated in the series.


1
2
3
4
5
A
Borate buffer
Running buffer
B
C
D
E
F
6
7
8
A
B
C



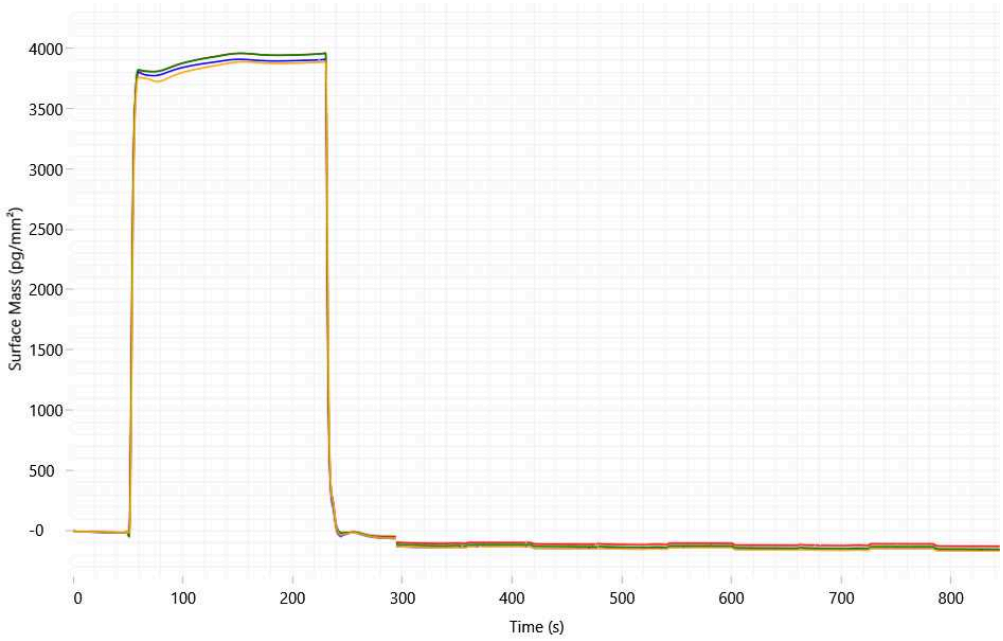
6.4 Go to the series and run the measurement.

6.5

	Step	Solution	Concentration	Injection duration	Flow rate (ul/min)
	Conditioning	Borate buffer	0.1M borate, 1M NaCl pH 9.0	180	10
	Wash (3x times)	Running buffer	1X buffer	60	100

Different steps in conditioning series.

6.6 Check the response and overall profile for the conditioning step.



Example profile for the chip conditioning step.

Note

Manual checks to determine if the chip is working:
1) The first injection with borate buffer gives a response between 3 000 - 4 000 pg/mm2
2) During the wash steps with running buffer the baseline should return to almost 0 pg/mm2 and be mostly flat.

Biotin (AVI) Tagged protein capture

7 Start short injection duration (i.e. 60 s) at low protein concentration to check the capture levels. Depending on the protein capture response, the concentration and injection duration can be increased.

7.1 In the **Measure** tab, choose **Immobilization** for the protein capture.

Screenshot of Creoptix software to perform immobilization

7.2 Select the type of immobilization as **Capturing biotinylated ligand to streptavidin surface**

7.3 Set the flow path to the appropriate *flow channel(s)* protein capture step e.g. FC2, FC3, or FC4.

FC = flow channel

7.4 Enter the details of the protein, concentration, and injection time in to the software.

The immobilization target level of the protein can also be added if known, but this is not required.

7.5 Click **Create Series**.

7.6 Go to Autosampler section in the method. Check all the solutions and their locations in the specified vial or plate.

7.7 Calculate the required immobilization level based on the equation given the below.

R_{max}=*(MW analyte/MW ligand) x Immobilized Ligand(pg/mm^2) x Stochiometry*

Immobilization level should be around less than 70 R_{max}.

7.8 Table 2. Biotin tagged protein capture method summary on the PCH-STA chip surface to check the protein capture level.

	A	B	C	D	E
	Wash (2x times)	Running buffer		60	10
	Capture	Biotin tagged protein	10 µg/mL	60	10

	A	B	C	D	E
	Wash	Running buffer		60	10

A: Step; B:Solutions; C: Concentrations; D:Injection Duration (s); E:Flow rate (uL/min)

7.9 Dilute the protein in the running buffer to the desired immobilization concentration. As a start, [M] 10 µg/µL protein solution can be tested to check capture response level.

7.10 Place the samples according to the series in autosampler.

7.11 Go to series in this method and start the capture step.

7.12 Check the capture protein response from the ***Measurements*** section in the project tree based on theoretical R_{max} calculation

Protocol references

1. Kartal Ö, Andres F, Lai MP, Nehme R, Cottier K. waveRAPID—A Robust Assay for High-Throughput Kinetic Screens with the Creoptix WAVEsystem. SLAS DISCOVERY: Advancing the Science of Drug Discovery. 2021;26(8):995-1003. doi:10.1177/24725552211013827.