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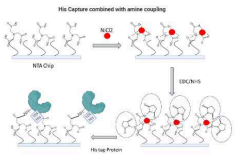
Creoptix Protein Immobilization Protocol: His-tag Capture via EDC/NHS chemistry



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

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Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abstract

This protocol outlines a comprehensive method for immobilizing His-tagged proteins on a PCH-NTA chip using the Creoptix WAVEsystem. The procedure involves chip conditioning, protein capture, and covalent immobilization through His capture and EDC/NHS chemistry. Key steps include surface activation with NiCl2, protein capture, and surface passivation with ethanolamine. It emphasizes the importance of optimizing protein concentration and injection duration to achieve desired immobilization levels. This method enables the stable attachment of His-tagged proteins in an oriented and active way for subsequent binding studies and is adaptable to various protein targets.

Materials

PCH-NTA: Pre-immobilized NTA Thick PC hydrogel **Malvern Panalytical Catalog #**9060009

Borate buffer: 0.1 M sodium borate, 1 M NaCl pH 9.0 **XanTec bioanalytics GmbH Catalog #**B BELU-50ML

EDTA: EDTA (0.5 M, pH 8.0, nuclease-free) **Thermo Fisher Scientific Catalog #**AM9260G

10x HBS-P: HBS-P Buffer 10× **Cytiva Catalog #**BR100671

EDC: EDC*HCl **XanTec bioanalytics GmbH Catalog #**C EDCHCL-1G

NHS: 0.1 M NHS pH 5.0 **XanTec bioanalytics GmbH Catalog #**B MN-50ML

$NiCl_2$:

1.option Nickel(II) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**223387

2.option NiCl2 loading buffer **XanTec bioanalytics GmbH Catalog #**B NILB-50ML

Ethanolamine:

Ethanolamine quenching buffer:1 M Ethanolamine * HCl pH 8.5 **XanTec bioanalytics GmbH Catalog #**B EA85-50ML

Safety warnings

Do not set the Flow Cell Temperature below 10 and/or 15°C. Lower temperature causes to the consensation in the chip.

Before start

The running buffer for each protein can be changed depending on the protein stability. Prior to start the method, a literature review should be done to check any background information on both protein stability and if any immobilization assay.

Instructions:

1 The running buffer for each protein can be changed depending on the protein stability. Prior to start the method, a literature review should be done to check any background information on both protein stability and if any immobilization assay.
Variables that are for the imobilization steps indicated the table.

	A	B
	Variable	Abbreviation for the variable
	Running buffer	**[X]**
	Protein concentration	**[Y]**
	Protein injection duration	**[Z]**

This information will be different depending on the specific protocol and protein target.

Setting up WAVEsystem

20m

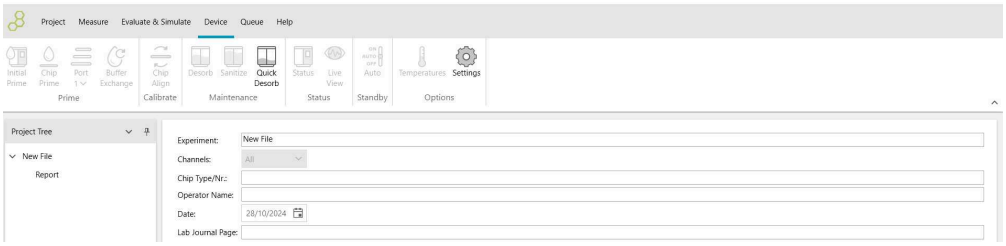
2 Dilute **10X running buffer [X]** to 1X reach a final volume of 500 mL with Miili-Q water. 20m

2.1 Degas the buffer for 00:02:00 in a ultrassonic bath. 2m

2.2 Keep 40 mL from the **1X running buffer** for preparation of samples and other solutions.

PCH-NTA chip conditioning

3 In the ***Device*** section, Start ***Priming*** and ***Chip Prime*** at least 2x.



Screenshot of Creoptix software to perform Prime and Chip prime

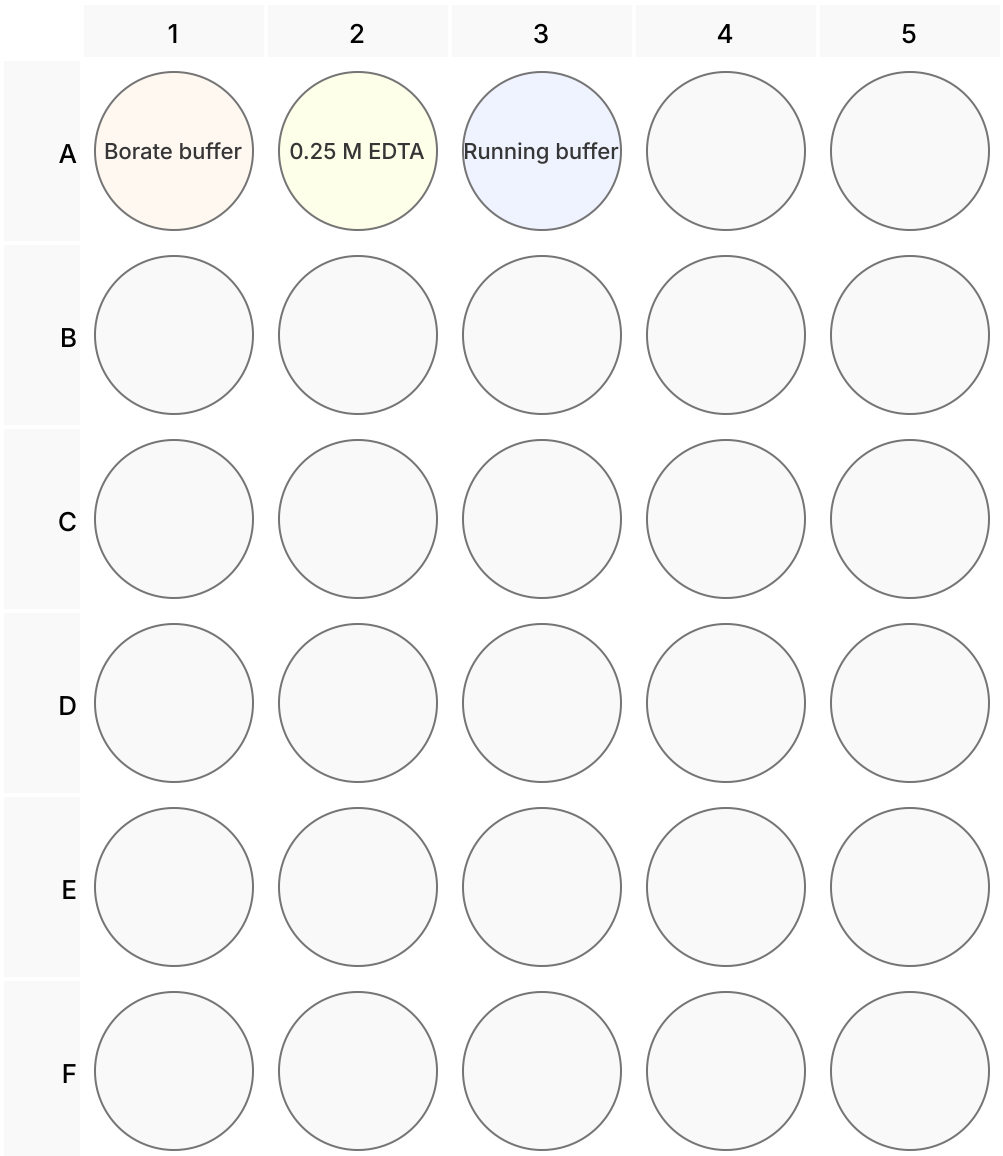
4 Conditioning of the chip is executed through wizard in ***Measure tab.***

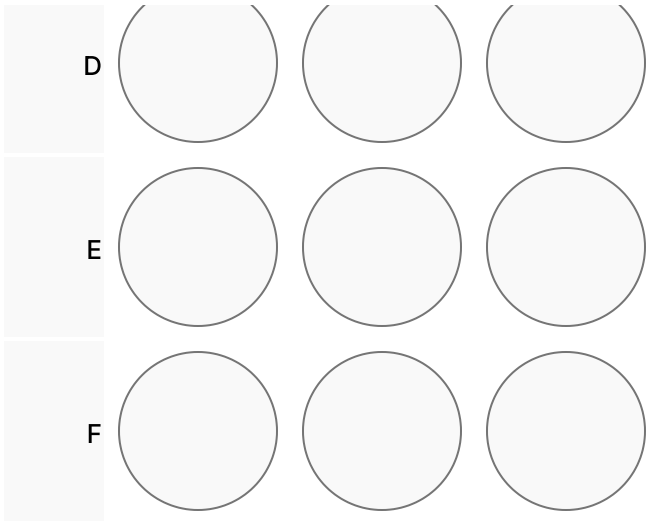
Screenshot of Creoptix software to perform Conditioning

4.1 Select chip type as **PCH-NTA** chip.

4.2 Define 1X running buffer.

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


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

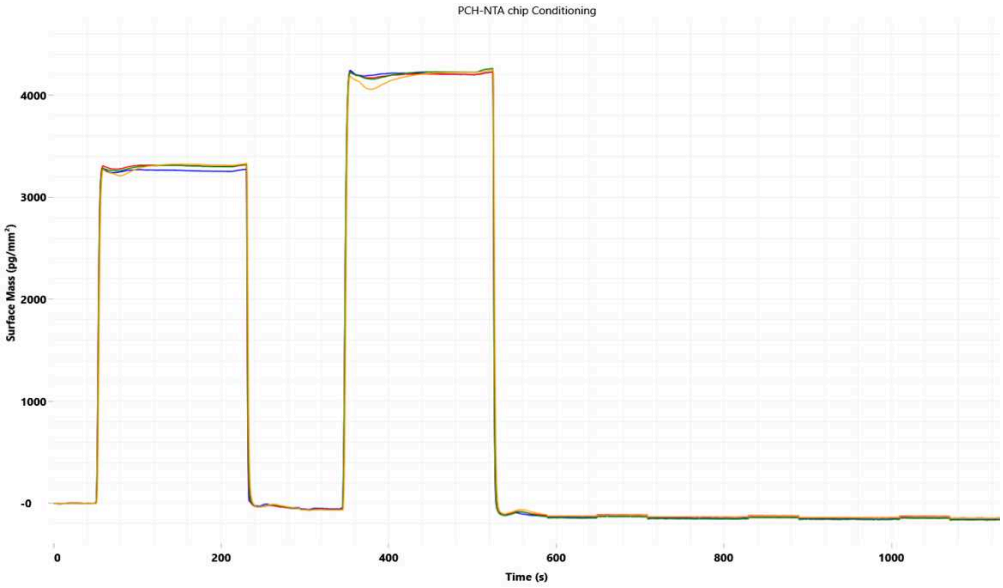


4.4 Go to the series and run the measurement.

5 A: Step, B: Solution, C: Concentration, D: Injection duration, E: Flow rate (uL/min)

	A	B	C	D	E
	Conditioning	Borate buffer	0.1M borate, 1M NaCl pH 9.0	180	10
	Conditioning	EDTA	250 mM	180	10
	Wash (3x times)	Running buffer	1X buffer	60	100

A: Step, B: Solution, C: Concentration, D: Injection duration, E: Flow rate (uL/min)



The graph after PCH-NTA chip conditioning

His Tagged protein immobilization

6 First, perform the capturing of His tagged ligands to Ni-NTA surface in the wizard (Table 2) to check whether the protein capture response at that concentration and duration is enough or not for the immobilization step.

6.1 Perform *Immobilization* assaywithin the **Measure** Tab.

Screenshot of Creoptix software to perform immobilization

6.2 First select the type of immobilization as ***Capturing His Tagged ligands to the Ni-NTA surface***

6.3 Select the Flow path as FC1 and FC2 for activation step.

6.4 Select FC2 for the capture step for His tagged protein and click create series. FC1 is used as reference “blank” channel. It should not have any protein in the chip.

6.5 Click *Create Series*.

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

6.7 Calculate the required immobilization level based on the equation given the below. Immobilization level should be around 70 to 100 Rmax.

7 Rmax=(MW analyte/MW ligand) x Immobilized Ligand(pg/mm^2) x Stochiometry

7.1 Table 2. His tagged protein capture method summary on the Ni-NTA activated surface to check the protein capture level.

	A	B	C	D	E
	Wash (2x times)	Running buffer		60	10
	Activation	NiCl2	500 uM	120	10
	Wash	Running buffer		60	10
	Capture	His tagged protein	20 ug/mL	420	10
	Wash	Running buffer		60	10
	Regeneration	EDTA	350 mM	60	10

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

7.2 Dilute the protein in the running buffer to final immobilization concentration in 250 µL uL final volume. As a start, [M] 15 µg/µL or [M] 20 µg/µL protein solution can be tested to check capture response level.

7.3 Prepare other solutions with 1X running buffer.

7.4 Place the samples according to the series in autosampler.

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

7.6 After the completion of this step, evaluate the capture protein response from the ***Measurements*** section in the project tree.

8

	A	B	C	D	E
	Activation	NiCl2	500 uM	420	10
	Wash (2x times)	Running buffer		60	10
	Activation	EDC/NHS	1:1 mix from 20C aliquots	420	10
	Capture	His tagged protein	[Y]	[Z]	10
	Passivation	Ethanolamine	1 M	420	10
	Wash	Running buffer		60	10
	Conditioning	EDTA	350 mM	420	10

A:Step, B:Solution, C:concentration, D:injection Duration, E:Flow rate (uL/min)

9 Once the evaluation and calculation are done, perform the immobilization assay for His tagged protein.

9.1 Perform Immobilization assay within the **Measure tab.**

9.2 First select the type of immobilization as *Capture and amine coupling of His-tagged ligands to the Ni-NTA surface.*

9.3 Select the Flow path as FC1 and FC2 (can be added FC3 and FC4 for different immobilization levels) for activation step.

9.4 Select FC2 for the Ni-NTA capture flow path and enter the details for protein concentration and injection duration(s)

9.5 FC1 is used as reference “blank” channel. It should not capture any protein.

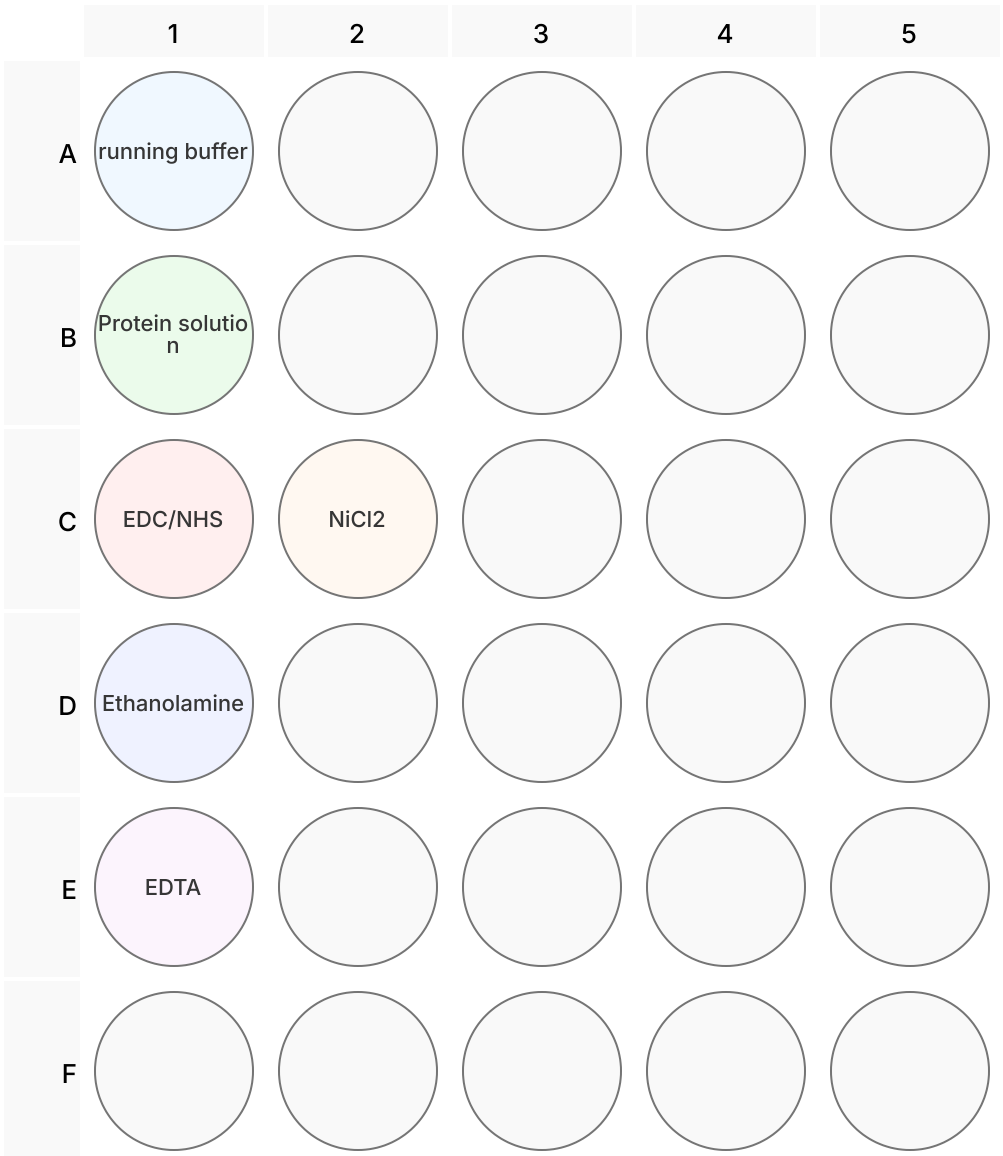
9.6 Select FC1 and FC2 paths for Passivation step (FC3 and FC4 if they are included) .

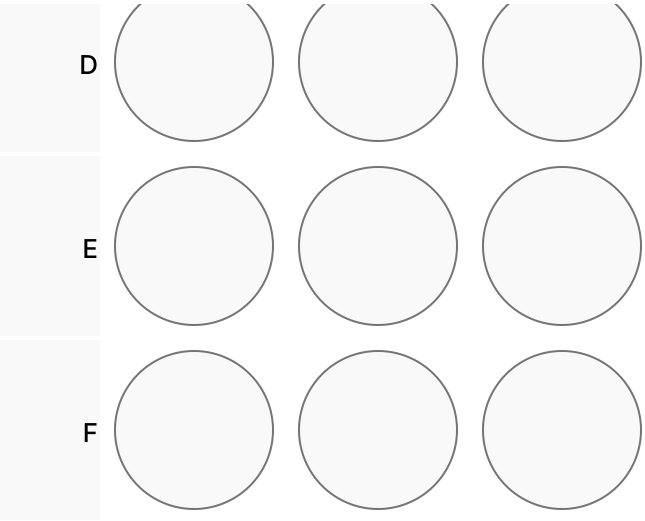
9.7 Click Create Series.

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

9.9 Go to Cycles tab in the method and check activation ($NiCl_2$ and EDC/NHS) and passivation, conditioning injections for only 1 and 2 otherwise all FCs are not included in the wizard. If they are included automatically, select only FC1 and FC2.

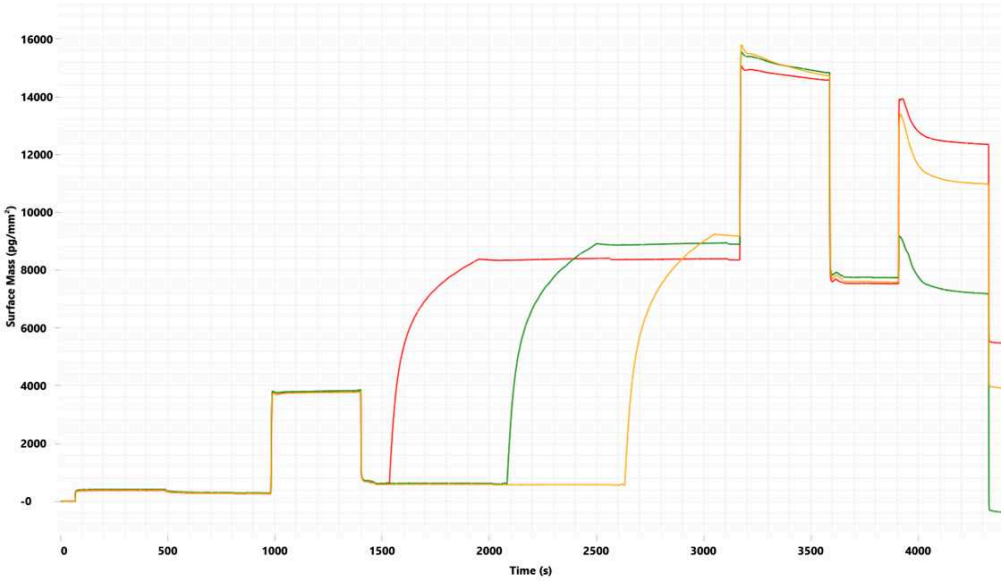
9.10 Place the samples according to the series in autosampler.


1
2
3
4
5
A
running buffer
B
Protein solution
C
EDC/NHS
NiCl2
D
Ethanolamine
E
EDTA
F
6
7
8
A
B
C



9.11 Go to series in this method and start the immobilization.

9.12 Check the sensogram in real time through Measurement tab in the Project Tree.



Immobilization sensogram with His capture +amine coupling strategy

9.13 Creoptix relies on refractive index changes. Even the running buffer is used to dilute and prepare the solution in the immobilization step, there are still some variations the immobilization step. The final response levels might indicate near zero and/or below

zero response as seen in the previous image (FC3:Green line). In this case, take protein capture level as a response.

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.
2. https://cdn.cytivalifesciences.com/api/public/content/digi-16083-pdf#:~:text=Capture%20of%20histidine%2Dtagged%20proteins%20for%20interaction%20analysis%20in%20Biacore,antibody%20to%20the%20sensor%20surface.

Acknowledgements

The immobilization flow figure is created in **BioRender.com**.