NHANCE™ PCS STARTER KIT

INNOVATION

The nHance™ Peptide Capture System (PCS) is a revolutionary, proprietary nanotechnology developed specifically for peptide sample preparation and quantification, eliminating many of the cumbersome steps associated with other approaches. The proprietary nHance™ coating at the heart of this system absorbs peptides within its nanopores based on size-exclusion, electrical charge and hydrophobicity. However, unlike conventional sample preparation technologies on the market today, the peptides are first captured and then subsequently released after washing in a quick and seamless elution step for subsequent bioanalytical analysis. Unwanted larger molecules and debris are removed during the washing procedures. Peptides of interest are extracted for subsequent identification and quantification absent of interference from the complex matrix.

MECHANISMS OF ACTION

The nHance™ PCS device is shown to be a multi-dimensional sample clean-up and small protein/peptide enrichment technique.

Electrostatic Interaction
Size Exclusion
Ion Pairing/Hydrophobicity

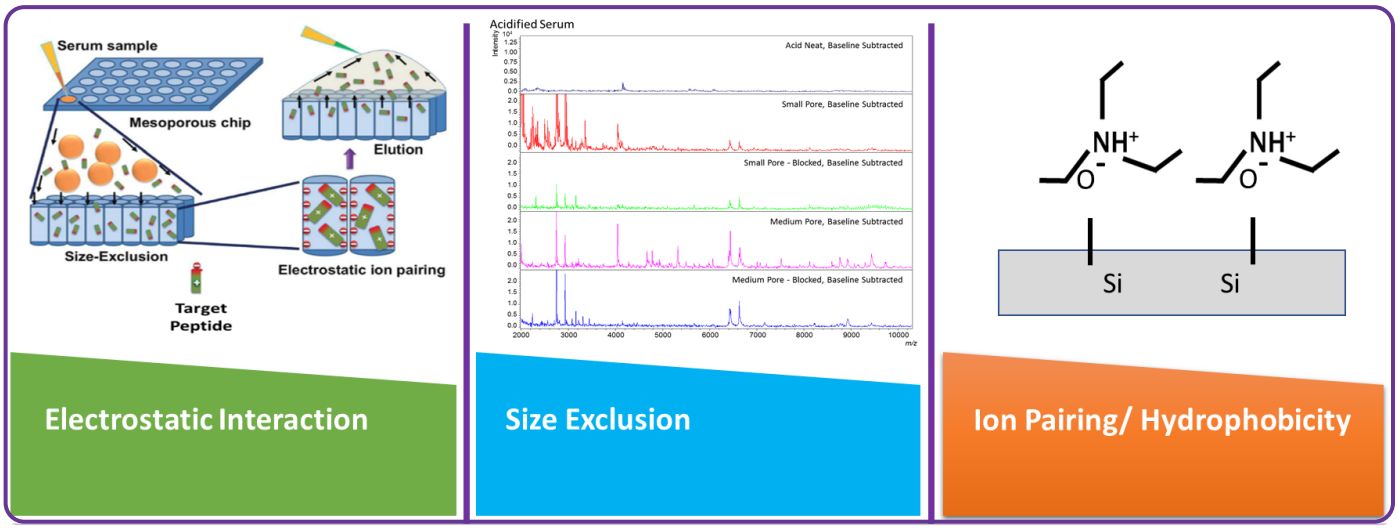

Figure 1: Mechanisms of Action

GENERAL NHANCE™ METHOD DEVELOPMENT PROCESS

Part I: Identify Peptide Parameters

Prior to using the nHance™ PCS 96-well plate, users are advised to acquire the following critical peptide parameters to maximize recovery and precision of their target molecule(s) of interest.

- Molecular weight (kDa)
- Iso-electric point (pI)
- pH vs peptide charge state

Part II: Plate Surface Blocking (Optional but Highly Suggested)

In order to avoid loss of the peptide of interest due to non-specific binding (NSB), it is recommended to block both the nHance™ PCS 96-well plate and collection plate prior to use. Several products are commercially available and are compatible with the nHance-PCS device.

Part III: Sample Pretreatment

Due to the negative charge state of the nHance-PCS pores, it is suggested that the pH of the sample be adjusted to elicit a neutral or positive charge state which is dependent on the pI of the peptide of interest. Although peptide specific, the suggested pH range of sample to be loaded onto the nHance PCS-device is between 1.5 to 11. See Table 1 for a list of potential acidic and basic solutions that can be employed to modulate the pH of the sample. For more information regarding pH Modulation, please consult the Application Notes found under the nHance™ website "Resources" page.

Part IV: Additional Pretreatments for Improved Peptide Recovery (Optional)

For highly complex biological matrices, such as serum or plasma, limitations due to loading capacity of a non-flow through system should be evaluated. Simple and common place sample pre-treatment processes have been employed in the nHance-PCS work flow to overcome loading capacity: protein precipitation and sample dilution/minimum required dilution. Example procedures are provided for guidance below.

STEP	PROCEDURE
1	Acidify sample solution, 10µL (1% Phosphoric Acid (PA) + 5% Acetonitrile (ACN)) to 90µL complex sample
2	Crash acidified sample with 150µL ACN
3	Vortex for 1 minute
4	Centrifuge sample for 5 minutes at 4000 rpm
5	Transfer supernatant to a new tube
6	Dry down tubes with Speed Vac for ~ 1 hour
7	Reconstitute in 50µL 1% PA + 5% ACN

Suggested Procedure 2: Protein Precipitation Procedure

STEP	PROCEDURE
1	Perform sample dilution: 1:10, 1:50, 1:100, 1:500.
2	Calculate % Recovery of peptide for each dilution.
3	Employ dilution with optimal % Recovery for future samples.

Sample Procedure 3: Sample Dilution Procedure (Minimum Required Dilution)

Load
Formic Acid (0.5 – 5 %)
Acetic Acid (0.5 – 5 %)
Phosphoric Acid (0.5 – 5 %)
Ammonium Acetate (5 – 50 mM)
Ammonium Formate (5 – 50 mM)
Trifluoroacetic Acid (0.5 – 5 %)
Ammonium Biocarbonate (5 – 50 mM)
Ammonium Carbonate (5 – 50 mM)
Acetonitrile (0 – 5%)
Methanol (0 – 5%)

Wash
Trifluoroacetic Acid (0.5 – 1.0 %)
1-O-octyl-β-glucopyranoside (10mM)

Elute
Ammonium Biocarbonate (5 – 50 mM)
Sodium Biocarbonate (5 – 50 mM)
Ammonium Carbonate (5 – 50 mM)
Ammonium Acetate (5 – 50 mM)
Ammonium Formate (5 – 50 mM)
Trifluoroacetic Acid (0.5 – 5 %)
Acetonitrile (35-100%)
Methanol (35-100%)
TRIS buffer (5-50 mM)

Table 1: List of reagents for nHance™ PCS procedures

NHANCE™ PCS WORKFLOW

The nHance™ PCS workflow consists of three critical steps: 1) Loading, 2) Washing, & 3) Elution.

Loading

This step consists of employing the appropriate pH sample modulation for your target molecule, and the introduction of an organic to "loosen" the peptide structure to encourage loading into the nHance™ nanoporous coating.

Wash

The washing steps are employed to remove any uncaptured proteins, salts, and other extraneous components commonly contained in complex biological matrices. Washing solutions are intended to remove unwanted sample material without impacting the retention of the target molecules loaded within the nHance nanoporous coating.

Elution

Peptide extraction is achieved by employing the same principals and strategy used when loading the peptides from a complex matrix. The elution solution will have the appropriate pH and organic modifier to elicit peptide release from the nHance™ material to promote optimal peptide recovery.

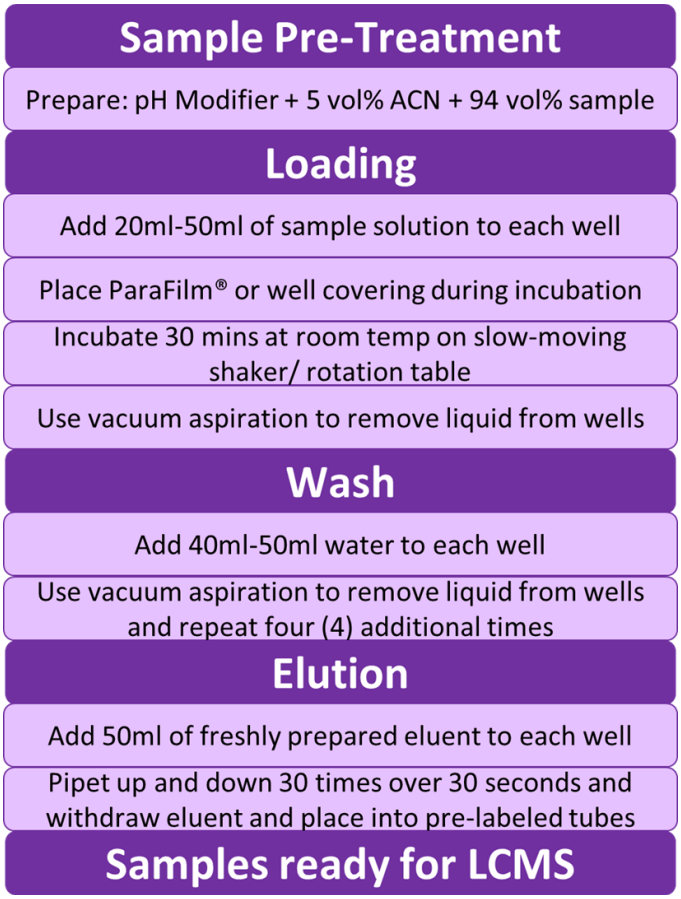

Figure 2: nHance™ PCS Workflow illustrating a simple 3-step process