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| **STANDARD OPERATING PROCEDURE** |
| |  |  | | --- | --- | | **Title: Peptide Immunoaffinity Enrichment** | | | **SOP#: M-01** |  | | **Version #: 1** | **Author: Paulovich Lab** | | **Date Approved:** | **Date Modified:** | |

1. PURPOSE

The purpose of this document is to describe peptide immunoaffinity enrichment for quantitative analysis compatible with mass spectrometry.

1. SCOPE

This procedure may be used to enrich peptides and stable isotope standards from digested samples.

1. RESPONSIBILITIES

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

1. Equipment
2. Materials
3. Reagents
4. Procedure
   * - remove plate from -80°C freezer and thaw
   * - centrifuge for 10min at 3000 RPM.
   * - place plate on a firm surface and carefully remove adhesive aluminum foil seal mat and silicone seal mat.
   * - inspect wells. Make note of any irregularities, missing samples, or other observations.

comments:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

* + - add 190 µL 1X PBS, 0.03% CHAPS and 10uL 1M Tris pH 8 into the wells 1 thru 90.
  + - mix well, care not to disturb the other lyophilized wells.
  + - Transfer samples to clean Kingfisher plates
  + -(optional if not performing immediate capture) seal plates with aluminum foil seal mat and freeze at -80C

**3) SISCAPA enrichment of analysis plates**

* + - remove one plate from -80°C freezer and thaw
  + - centrifuge for 10min at 3000 RPM.
  + - place plate on a firm surface and carefully remove adhesive aluminum foil seal mat.
  + - check pH by pipetting 1uL onto a pH 5-10 test strip (EMD Chemicals pH 5-10 colorpHast test Strips No. EMD-9588-3. Verify that pH is between pH 7.5 – pH 8.0.
  + - prepare ‘Control Capture mix’ (CCMIX). 1X PBS, 0.03% CHAPS, digested plasma (Bioreclamation # HMPLEDTA2) heavy peptides at 0.5 fmol/µL, as described in the table below:

Note: this is prepared in bulk and is used as a quality control standard.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Control Capture SISMIX Table** | | | | **for 30 capture plates** |
| **Component** | **MUX Stock Conc** | **Final Conc** | **DF** | **volume (µL)** |
| **Heavy peptide (13C/15N) MUX (fmol/ul)** | 1000 | 0.5 | 2000 | 10 |
| **Digested plasma (labeled 0.5mL equiv. resuspended in 0.5mL 1X PBS, 0.03%CHAPS)** | 500 | 500ul/600ul | 1 | 16700 |
| **1X PBS, 0.03%CHAPS** | 1x | 1x | 1 | 3290 |
| **Final Volume (ul)** |  |  |  | 20000 |

* + - add 200 µL of the ‘Control Capture’ mix (CCMIX) and 10uL 1M Tris pH 8 i into wells 91, 92, 93.
  + - check pH 7.5-8.0
  + - prepare the antibody bead mastermix

Note: Mastermixes may be made up the day before use and stored at 4oC.

* + - add 50 µL of the cross-linked antibody bead mastermix to wells 1- 93 on the plate.
  + - cover the plate with adhesive foil seal. Make sure there is a good seal.
  + - place plate on Labquake mixer using rubber bands, Velcro strips, or ties and mix by slowing inverting overnight (12-16h) at 4oC.

NEXT DAY

* + - the next day, install the PCR magnet head on the Kingfisher bead handling platform, prepare the following plates and load the Kingfisher method *SISCAPA\_Apr2011.kf2 (this may be adjusted if a newer version (3.1) of software is installed (instead of ver 2.6).*

Plate 1: incubation plate (~250 µL)

Plate 2: 250 µL1XPBS 0.01%CHAPS

Plate 3: 250 µL 1XPBS 0.01%CHAPS

Plate 4: 250 µL 0.1X PBS 0.01% CHAPS

Plate 5: 25µL 3% acetonitrile:5% acetic acid

Plate 6: bead collection plate, 100µL PBS 0.03% CHAPS, 0.1% sodium azide

Plate 7: tip comb

* + - remove plate from Labquake and centrifuge at 3000 RPM for 30-60 seconds to remove liquid that may be on the seal surface.
  + - carefully remove the foil seal and place the incubation plate in position 1 on the Kingfisher and start the method.

Kingfisher steps:

- wash the beads 2 times with 250 μL PBS + 0.01% CHAPS (1.5 min per wash).

- wash the beads 1 time with 250 μL 0.1X PBS + 0.01% CHAPS (1.5 min per wash).

- elute the peptides in 25 μL of 3% acetonitrile:5% acetic acid (5 min).

- collect used beads into a fresh collection plate (5 min).

*As per protocol outlined in the Kingfisher method,* ***SISCAPA\_Apr2011.kf2****, beads are transferred to a fresh collection plate after the elution step (plate 6).*

* + - when the Kingfisher method has finished, seal the incubation plate (plate 1) and the collected antibody bead plate (plate 6) with adhesive foil and store at -80°C and 4°C, respectively.
  + - remove elution plate (Plate 5) from the KingFisher and transfer into daughter analysis plates as described below:

**4) Preparation of Samples for LC-MRM Analysis**

* + - label 3 new PCR plates with appropriate name and add 5 µL of 3% ACN:0.1%FA to the wells designated to contain sample.
  + - place the elution plate on a magnet plate.
  + - using a multi-channel pipet, draw the eluate of 10 patients (30 wells) to each plate without touching the bottom of the well and transfer into corresponding wells of the new PCR well plates (refer to plate layouts below).
  + -transfer one CCMIX replicate to each of the analysis plates
  + - cover the plates with aluminum foil and place in -80C.
  + - when ready for analysis, remove analysis plate, thaw (if necessary), spin the plate at 3000 rpm for 30-60seconds.
  + Add MS QC standards, blank injections (3% ACN:5% acetic acid), and wash solvents to regions of the plate not containing samples (usually wells 94-96)
  + - cover the plates with silicon seal mat and place on magnet plate on the autosampler.
  + - analyze by LC-MRM using the appropriate method.

1. Referenced Documents