STANDARD OPERATING PROCEDURE

Title: Liquid Chromatography, Eksigent NanoFlex, trap and elute

Version #: 1   **Author: Paulovich lab**

Date: 8/17/2015

Purpose

The purpose of this document is to describe the liquid chromatography (LC) method for quantitative analysis.

Scope

This procedure encompasses the setup of the LC and method parameters. It is specific to operation of the dual column chip system using an Eksigent Ultra system coupled to nanoflex cHiPLC.

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.

Equipment

- HPLC: NanoLC-2D plus (Eksigent, 950-00061)
- Eksigent NanoFlex chip system.

Materials

- Injection loop: 1 uL peeksil, 100 um (Eksigent, 200-00208)
- Column Compartment: cHiPLC-nanoflex (Eksigent, 950-00070)
- Trap column: 200 μm x 0.5 cm ChromXP C18-CL 3 μm 120 Å (Eksigent, 804-00006)
- Analytical column: 75 μm x 15 cm ChromXP C18-CL 3 μm 120 Å (Eksigent, 804-00001)
- Water, HPLC grade (H2O) (Fisher, W5-1)
- Acetonitrile, HPLC grade (ACN) (Fisher, A998-1)

- Formic Acid (FA) (EDM, 11670-1)

Solutions

Mobile phases. Must be degassed every week:
- Gradient 1, mobile phase A: 0.1% FA in H2O
- Gradient 1, mobile phase B: 0.1% FA in 90% ACN
- Gradient 2, mobile phase A: 0.1% FA in H2O
- Gradient 2, mobile phase A: 0.1% FA in 90% ACN)

Procedure

1. Autosampler method:

Step #	Operation	Value	Parameter	Speed	Height	Description
1	Output		1-OFF			Wait for Gradient 1 ready to start
2	Output		2-OFF			Wait for Gradient 2 ready to start
3	Valve		Injector Load			Valve Position Control
4	Aspirate	10.5	Reagent-1	1	3	Aspirate specified volume
5	Wait	00:00:05				Pause for specified time
6	Aspirate	0	Reagent-1	1	3	Aspirate specified volume
7	Aspirate	4.2	Sample	1	9	Aspirate specified volume
8	Wait	00:00:05				Pause for specified time
9	Aspirate	0	Sample	1	10	Aspirate specified volume
10	Aspirate	0	Reagent-3	1	5	Aspirate specified volume
11	Aspirate	5.3	Reagent-1	1	5	Aspirate specified volume
12	Wait	00:00:05				Pause for specified time
13	Output		1-ON			Start LC run on channel 1
14	Output		2-ON			Start LC run on channel 2
15	Valve		Injector Inject			Switch AS injector valve to Inject position (1-2)
16	Marker		Inject			Pause for specified time
17	Wait	00:10:00				Pause for specified time
18	Valve		Injector Inject			Switch AS injector valve to Load position (1-6)
19	Dispense	20	Waste	3	0	Dispense specified volume
20	Needle Wash	50	Port 1			Perform needle wash
21	END					

2. Gradient 1 method:
    i. Flow rate (μL/min): See timetable
    ii. Temperature (C): 40
    iii. Run Conditions:
        a. Pre-run
        b. Flush column for 0.1 minutes using 100% initial flowrate conditions.
    iv. Timetable

Time (min)	% Mobile phase A composition	% Mobile phase B composition	Flow rate (µL/min)	Event
0	99	1	10	Valve Load
2	99	1	10	Valve Inject
2.1	50	50	2	
65	50	50	2	
65.1	99	1	10	
75	99	1	10	Valve Load
50	99	1	10	

3. Gradient 2 method:
   i. Flow rate (nL/min): 300
   ii. Temperature (C): 40
   iii. Run Conditions:
      a. Pre-run
      b. Flush column for 0.1 minutes using 100% initial flowrate conditions.
   iv. Timetable for column 1 elution:

Time (min)	% Mobile phase A composition	% Mobile phase B composition	Event
0	99	1	
3	99	1	
10	90	10	
40	75	25	
55	60	40	
65	40	60	
66	10	90	
69	10	90	
70	99	1	
70	99	1	

http://proteomics.cancer.gov