

iGEM 2016 – SDU	
Title: C8 and C18 columns	Date issued: 2016.10.04
SOP number: SOP0039	Review date: 2016.10.11
Version number: v1	Written by: Joel Vej-Nielsen

1. Purpose

Reversed-phase R2/R3 micro purification of peptides

2. Area of application

This procedure is valid for prepared cell digests.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Syringe P200 pipette tips			

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
C8 membrane				
C18 membrane				
R2 reverse-phase resin				
R3 reverse-phase resin				
Acetonitril	100%			
Acetonitril	0.1%			
Elution buffer	60% acetonitril 0.1% TFA			

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0037 - iTRAQ sample preparation

SOP0038 – Qubit® Protein Assay Kits

SOP0040 - iTRAQ labelling

SOP0041 - TiO₂ purification

7. Environmental conditions required

8. Procedure

- 8.1 Cut pipette tips, so they fit to syringe without letting air out and combine the two.
- 8.2 Add C8/C18 to another p200 pipette tip, make sure no liquid can pass through the tip around the material.
- 8.3 Mix R2/R3 with 100% acetonitrile.
- 8.4 Pass appropriate amount of R2/R3 solution through the column by adding to the C8/C18 material and putting putting the syringe with the tip inside the pipette tip and lightly applying pressure on the syringe. (pipette up and down in order to mix before pipetting the solution).
- 8.5 Wash filter twice with 120 µl 0.1% acetonitrile by same method, avoid letting the sample dry by not pressing all the liquid through the column.
- 8.6 Pass sample through column.
- 8.7 Wash filter twice with 120 µl 0,1% acetonitrile
- 8.8 Elute sample with 60 µl elutionbuffer

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks

10. Time consumption

- Total-time 45 m.
- Hands-on-time 20 m.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.10.04 / JVN	01	The SOP has been written
16.10.11 / JR		The SOP has been revised

12. Appendixes