iGEM 2016 – SDU

Title: TiO_2 purification	**Date issued:** 2016.10.04
SOP number: SOP0041	**Review date:** 2016.10.11
Version number: 01	**Written by:** Joel Vej-Nielsen

1. Purpose

Procedure for isolation and enrichment of phosphopeptides in samples.

2. Area of application

This procedure is valid for digested protein samples.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
SpeedyVac			
Vortex			
Centrifuge			
scale			

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Loading Buffer	80% Acetonitril, 5% TFA & 1M glycolytic acid (76 mg/ml)			
Washing Buffer 1	80% Acetonitril & 1% TFA			
Washing Buffer 2	10% Acetonitril & 0,1% TFA			
Elution Buffer	60µl ammonia solution in 940µl H_2O (pH 11,3)			
TFA	0,1%			
TFA	10%			
TFA	100%			
Acetonitril	30%			
HCl	12M			
HCl	1M			
PNGase F				
Sialidase A				
Formic acid	100%			
Low binding eppendorftubes				
$\boldsymbol{TiO_2}$ beads				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0037 - iTRAQ sample preparation

SOP0038 – Qubit® Protein Assay Kits

SOP0039 – C8 and C16 column purification

SOP0040 - iTRAQ labelling

7. Environmental conditions required

8. Procedure

8.1 Mix the TiO_2 loading buffer in your protein sample. (Add water, TFA Acetonitrile and glycolic acid in that order.
8.2 Add 0.6 mg TiO_2 beads pr. 100 µg initial peptide solution.
8.3 Incubate on shaker (highest setting) for 15 minutes at room temperature.
8.4 Centrifuge sample briefly and move supernatant to another low binding Eppendorf tube with half the amount of TiO2 beads used previously.
8.5 Incubate for sample for 10 min. On shaker (highest setting).
8.6 Centrifuge briefly and move supernatant to another low binding Eppendorf tube (Mark tube "Non-modified peptides").
8.7 Pool beads from both incubations with 100 µl binding buffer and transfer to new low binding Eppendorf tube
8.8 Vortex for 10 sec. and centrifuge briefly, before collecting supernatant in "Non-modifed peptides" tube.
8.9 Wash beads with 100µl washing buffer 1, mix for 10 sec, centrifuge and move supernatant to "Non-modifed peptides" tube
8.10 Wash beads with 100µl washing buffer 2, mix for 10 sec, centrifuge and move supernatant a new Eppendorf tube. (Mark tube "TiO_2 wash2")
8.11 Dry the TiO_2 beads in vacuum centrifuge for 5-10 min.
8.12 Prepare 150 µl elution buffer.
8.13 Elute phosphopeptides by adding 150µl elution buffer and leave on shaker for 15. Min.
8.14 Centrifuge solution for 1 min. (highest speed) pass the supernatant over a filter (C8 stage tip) to recover liquid in a low binding tube.
8.15 Wash beads with 30µl elution buffer and pool supernatant with the other supernatant that has been filtered.

8.16 Elute from C8 filter with 5 µl 30% acetonitrile
8.17 Add 7,3 µl HCl (12 M) and adjust the pH of the solution to 7,5 with 1 M HCl solution.
8.18 Add 1 µl PNGase F & 0.5 µl sialidase A to the solution and incubate for one hour at 37°C.
8.19 Add 10 µl 100% formic acid and 5 µl 10% TFA to acidify solution.
8.20 Purify peptides by using two p200 R3 micro columns sequentially.
8.21 Wash columns with 60µl 0,1% TFA and elude phosphoproteins with 60 µl 60% acetonitrile, 0,1% TFA. Start with the second column then the first one and then the eluate is collected in a new Eppendorf tubed (mark tube "TiO_2-R3-eluate".
8.22 Run a MALDIon 0,5 µl sample and a LC-MS/MS on 5 µl for individual group run.
8.23 Dry the samples by vacuum centrifugation
8.24 Lyophilize cells.

9. Waste handling

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

10. Time consumption

- Total-time 5h
- Hands-on-time 3h

11. Scheme of development

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

12. Appendixes