iGEM 2016 – SDU

Title: Sample preparation	**Date issued:** 2016.10.04
SOP number: SOP0037	**Review date:** 2016.10.11
Version number: 01	**Written by:** Joel Vej-Nielsen

1. Purpose

Digesting cell pellet for phosphoprotein purification

2. Area of application

This procedure is valid for all *E. coli* strains.

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Vacuum centrifuge			
Probe sonicator			
50ml falcon tubes			
Eppendorf tubes			
Centrifuge			
P20, P200 & P1000 pipettes			

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

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
PBS				
Lysis buffer (100µl)	93,8 µl Urea (6M) thiourea (2M) solution 1 µl 1M DTT (10mM) 3 µl phosSTOP phosphatase inhibitor 1,2 µl complete protease inhibitor 2 µl benzonase	Sigma Aldrich		Handle in fume closet
Triethyla-mmonium bicarbonate (TEAB)	20mM, pH 7,5			
Iodoacetamide				
Trypsin				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

SOP0001 – ON culture
SOP0038 – Qubit® Protein Assay Kits
SOP0039 – C8 and C16 column purification
SOP0040 - iTRAQ labelling
SOP0041 - TiO2 purification

7. Environmental conditions required

8. Procedure

8.1 Prepare 50 ml cell culture of relevant bacterial strains.
8.2 Grow the cell cultures to an OD_{600}= 0.4-0.5.
8.3 Move 45 ml to a 50 ml falcon tube
8.4 Centrifuge the cell cultures for 5 min. at 4000 rpm and discard supernatant.
8.5 Resuspend cells in 10 ml PBS.
8.6 Centrifuge solution at 4000 rpm for 5 min. And discard the supernatant.
8.7 Resuspend cells in 5 ml PBS.
8.8 Centrifuge solution at 4000 rpm for 5 min. And discard the supernatant.
8.9 Resuspend cell pellet in 1ml PBS and move to Eppendorf tube.
8.10 Centrifuge the cell cultures for 5 min. at 4000 rpm and discard supernatant.
8.11 Store cells at -80℃.
8.12 Resuspend appropriate amount of cell pellet in 15 µl lysis buffer.
8.13 Incubate for 30 min. at room temperature.
8.14 Add 135 µl TEAB.
8.15 Keep samples on ice and sonicate for 2x10 seconds.
8.16 Measure protein concentrations from 1 µl of each sample on Qubit.
8.17 Add iodoacetamide to a final concentration of 20 mM and incubate for 30 min. in the dark.
8.18 Add 1 µg of trypsin pr. 30 µg protein and incubate over night at room temperature.
8.19 Dry down cells in speedy vac.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Supernatant		Liquid bacterial waste	
Once use plastic		GMO yellow waste	

10. Time consumption

- Total-time 9h.
- Hands-on-time 1h.

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	01	The SOP has been reviewed

12. Appendixes