# Protein Extraction Protocol

## Introduction

This protocol is an adaptation of a GUS (β-glucuranidase) assay provided by Zofia Dorota Jarczynska and adapted by Jacob Mejlsted

## Materials

### Extraction buffer (1L)

- 50 mM $Na_3PO_4$, pH 7 (50 mL of 1 M stock solution)
- 10 mM β-mercaptoethanol (0.7 mL of 14.4 M stock solution)
- 10 mM $Na_2EDTA$ (20 mL of 0.5 M $Na_2EDTA$ stock solution)
- 0.1% Sodium Lauryl Saccosine (10 mL of 10% Sarcosyl)
- 0.1% Triton X-100 (10 mL f 10% Triton)
- MilliQ water up to 1L

### Equipment

- Centrifuge with cooling
- Plate reader
- Homogenizer
- Beads (big, metal)
- Sterile funnels with mira cloth

### Consumables

- Liquid nitrogen
- Microtiterplate
- FastPrep tubes

### Chemicals

- Bradford reagent (like SigmaAldrich B6916)
- BSA standard (Bovine Serum Albumin 2 mg/mL)

## Procedure

### Protein Extraction

1. CRITICAL Set the centrifuge at 4°C
2. Seperate biomass from supernantant using mira cloth or other methods
3. Take ~100mg biomass and place in a FastPrep tube with one big metal bead
4. Place the tubes in liquid nitrogen

5. Homogenize for 1 min at 45 Hz
6. Add 500 µl extraction buffer
7. Homogenize for 2 min at 45 Hz
8. Centrifuge for 2 min at 10,000 x g at 4°C
9. Collect the liquid phase and place in a new tube
10. Centrifuge for 15 min at 10,000 x g at 4°C
11. Aliquote the supernantant into new tubes
12. Store at -80°C

## Fluorescence Assay (optional)

13. Transfer 100 µl protein sample into a microtiter place
14. Measure fluorescense at the appripriate wavelenghts

    Like (Ex/Em) 584/697 for mRFP1

## Bradford Assay

15. Mix 20 µl protein sample with 230 µl Bradfor reagent
16. Transfer to a new microtiter plate
17. Measure the blue color at 595 nm

    **Remember to include a standard curve and Bradford reagent**
18. Determine protein concentration