SCyCode – PROTOCOL

Cell harvest for (phospho)proteome analysis

Established by: P. Spät, July 2013
Reference: Spät et al., 2015

EQUIPMENT

- 250 or 500 mL Centrifugation flasks
- Centrifuge (Requirements: cooling to 4 °C; 7,500 RCF)
- 50 mL falcon tubes

PROCEDURE

Cyanobacteria cultures are rapidly cooled down to reduce enzymatic activity. Therefore, centrifuge flasks are prepared with ice and cooled to -20 °C before cultures are added and cells are pelleted by centrifugation.

PREPARATION

1. For 500 mL centrifugation flasks, add approx. 130 g crushed ice and freeze at -20 °C.
   (adapt a similar ice:culture ratio for smaller volumes)
2. Pre-cool centrifuge and rotor (0-4°C)

CELL HARVEST

3. Add approx. 300 mL of the culture to the pre-cooled flask and mix by gentle shaking
   (only a small portion of ice should remain)
4. Centrifugation for 8 min with 7,500 RCF at 4°C
5. Remove the clear supernatant carefully
6. Resuspend cell pellet in 30 mL ice-cold PBS buffer or BG11 medium
7. Transfer cell solution into 50 mL falcons on ice
8. Centrifugation for 10 min with approx. 3,500 RCF (or more) at 4°C
9. Remove the clear supernatant carefully
10. Snap freeze the cell pellet in liquid nitrogen (storage at -80°C)

REFERENCE

1. Spät, P., Macek, B., and Forchhammer, K. (2015) Phosphoproteome of the cyanobacterium *Synechocystis* sp. PCC 6803 and its dynamics during nitrogen starvation. *Frontiers in Microbiology* **6**