**Generation of uniformly 13C-labelled *E. coli* extract**

**Materials**

* *E. coli* – MG1655 strain
* 2L flask
* 2× Centrifuge bottle (750ml)
* 2× 50ml tube
* M9 minimal medium
* Extraction solvent (ACN:MeOH:H2O=2:2:1)
* Liquid nitrogen (lq. N2)
* 13C-Glucose

**Equipment:**

* Suitable container for lq. N2
* pre-cooled centrifuge to 4 °C

**Procedure**

1. Grow *E. coli* cells in 2 × 500ml of M9 minimal medium with 0.2% of 13C glucose at a shaking incubator at 37°C overnight
2. Measure optical densities (O.D. = approximately 1)
3. Centrifugation at 3000×g for 10 min
4. Remove supernatant (don’t remove all) and resuspend in M9 minimal medium
5. Transfer cell solution into 50ml tube
6. Centrifugation at 3000×g for 10 min
7. Remove supernatant and add 6ml of ice cold extraction solvent (stored at -80°C before use)
8. Freeze in lq. N2 for 1min and votex for 30s (repeat this freeze-thaw extraction 4 times)
9. Centrifugation at maximum speed for 10min
10. Take supernatant and apply argon gas to sample tubes
11. Store at -80°C