

## Lysis Method Comparison

**Goal:** Compare various lysis methods for the determination of intracellular NADPH and NADH in *E. Coli BL21* with and without the *zwf* gene.

**Background:** Last week, we compared various sonication methods by determining the fluorescence of mCherry protein before and after sonication. However, when the methods were applied to tests for NADPH and NADH, the levels of each were negligible. We believe that the sonication may have destroyed the NADPH and NADH in the solution.

### Comments:

- All samples should be kept on ice at all time.
- Readings should be done immediately.

### Protocol:

1. Prepare starters of *E. Coli BL21* with plasmid pSB1C3 containing pT7 and RBS, as well as *E. Coli BL21* with plasmid pSB1C3 containing pT7, RBS, and the *zwf* gene. Don't forget to add CM antibiotic! Incubate at 37 Celsius in the shaker, overnight.
2. Dilute the starters at a ratio of 1:100 and incubate in the shaker until O.D. at 600 nm is 0.6.
3. Pipette 1 ml of samples into 27 halicots (3 lysis techniques x 3 settings x 3 samples)
4. Centrifuge at the maximum speed for 5 minutes. Discard supernatant.
5. Quench cells twice with methanol on liquid nitrogen.
6. Resuspend cells in 0.5 ml cold BA.
7. Freeze in liquid nitrogen until use.
8. Conduct the relevant lysis method (as listed in the tables below)
9. Read wells in fluorescence reader with 340 nm excitation and 460 nm emission.

### Freeze-thawing:

Sample #	Time in -30 C freezer (min)	Time in liquid nitrogen (min)	Time in water bath (min)	Water bath Temperature (C)	Repeats
1	10	0	10	30	1
2	0	3/4	15	30	3
3	10	0	10	30	2

