

Auto-induction protein expression

ZYP auto-induction media:

- N-Z-Amine (3 g)
- yeast extract (1.5 g)
- 1M K_2HPO_4 (15 mL)
- 1M KH_2PO_4 (15 mL)
- 1M $(NH_4)_2SO_4$ (7.5 mL)
- dH_2O (to 300 mL)

This mix has to be autoclaved, then when the media is cold add:

- 1M $MgSO_4$ (0,6 mL) *sterilized by filtration (0.22 μm)
- 1000x trace element solution (0,6 mL)
consists of: $FeCl_2$ (50 mM), $CaCl_2$ (20 mM), $MnCl_2$ (10 mM), $ZnSO_4$ (10 mM),
 $CoCl_2$ (2 mM), $CuCl_2$ (2 mM), $NiCl_2$ (2 mM), HCl (60 mM), Na_2MoO_4 (2 mM),
 Na_2SeO_4 (2 mM) and H_3BO_3 (2 mM) *sterilized by filtration (0.22 μm)
- 50x 5052 solution (6 mL)
consists of: glycerol (25 g), glucose (2,5 g), α -lactose monohydrate (10 g) and dH_2O (to 100 mL) *sterilized by filtration (0.22 μm)
- antibiotic (50-100 $\mu g/mL$)

For an easier in-house media, a powder mix could be prepared. For #100 300mL batches, mix:

- 120 g N-Z-Amine
- 60 g yeast extract (1.5 g)
- 104 g K_2HPO_4 (15 mL)
- 82 g KH_2PO_4 (15 mL)
- 39 g $(NH_4)_2SO_4$ (7.5 mL)

Therefore, weight **9.1 g** of such mixture and add 293 mL of water. Autoclave and then add $MgSO_4$, 1000x trace element, 50x 5052 solution, and antibiotic as reported before.

expression:

- pick a single colony from a LB plate and inoculate in the flask containing the media
- leave under shaking 200 rpm at the wanted temperature.

Normally 37 °C for 20-24 hours are acceptable condition for a good expression of many common proteins, otherwise the system could be optimized. For instance, 8 hours at 37 °C (when the glucose is feeding the cells and there is not protein expression) and then decrease to 28 °C for other 20 hours to allow the expression.