3α-HSD Activity Measurement Protocol

Goal: verification of enzymatic activity of 3α-HSD by measurement of decrease in NADPH fluorescence over time in presence of dihydrotestosterone (DHT).

Protocol:

Preparation:

1. **The day before:** prepare starters in LB (5ml with 5µl antibiotics) and place in shaker overnight at 37℃ (16-20hr)
2. Dilute the bacteria 1/100 (50ml LB + 50µl antibiotics) in a 250ml Erlenmeyer and check O.D. at 600 nm.
3. Grow culture until it reaches O.D.(600nm) = 0.6.
4. Transfer to 50ml falcon and centrifuge for 5 min at 5000rpm. Re-suspend the pellet in BA (bioassay) medium.
5. Add 0.1mM IPTG (5µl) and place in shaker for 2-3 hr.
6. Transfer 1ml into Eppendorf tube and centrifuge for 5 min at 5000rpm.
7. Re-suspend the pellet in 1ml phosphate buffer (PBS) pH=7.4.
8. Sonicate at 20% amplitude for 3 cycles of: 5 sec sonication and 30 sec on ice.
9. Centrifuge for 10 minute at max speed.

Reaction:

1. Keep 96-well microplate on ice and add 170µl of the cell lysate supernatant.
2. Add 150µM NADPH to each plate as fast as possible to minimize degradation.
3. Place plate in plate reader for 30min at 37℃for stabilization. Use kinetic measurement program to measure fluorescence every 1 minute.
4. Take out plate and add 50µM DHT to each plate using multi-pipette.
5. Place plate in plate reader for 3hr at 37℃and measure every 1 minute.

Comments:

1. For NADPH We used 1.5mM stock solution, made by dilution of powder purchased from Sigma-Aldrich in 0.01N NaOH. Link to product: https://www.sigmaaldrich.com/catalog/product/sigma/n1630?lang=en&region=IL

2. For DHT we used 1mM stock solution, made by dilution of 1mg/ml solution purchased from Sigma-Aldrich in methanol. Link to product: http://www.sigmaaldrich.com/catalog/product/cerillian/d073?lang=en&region=IL

3. Fluorescence measurement was performed in Tecan-infinite 200Pro plate reader, using 340nm excitation and 460nm emission filter.

4. Controls: no 3αHSD gene, no lysate, no DHT, no NADPH**.**

Written by Ruth, Alexey and Adi