Endo-β-galactosidase assay (Detection of sugars after EBG digestion)

Aim

To detect the presence of sugars released from a substrate after digestion with endo-β-galactosidase.

Procedure

ENDO-β-GALACTOSIDASE DIGESTION

1. Add 30 µl of PGM stock solution (10 µg/µl) into an 1.5 ml eppendorf tube **(Total protein: 300 µg)**.
2. Add 27 µl of reaction buffer.
3. Add 3 µl of stock enzyme solution.
4. Incubate overnight at 37ºC

CENTRIFUGE THE DIGESTED PRODUCT (Amicon Ultra 0.5 Centrifugal Filter 10K Device)

1. Insert the filter into one of the provided micro centrifuge tubes.
2. Add sample in the filter.
3. Wash the eppendorf where the digestion took place with 60 µl reaction buffer.
4. *Note: Total volume per sample 120 µl. PGM concentration per sample: 2.5 µg/µl.*
5. Add it to the filter and cap it.
6. Place the capped filter device into the centrifuge. *Note: Align the cap strap toward the center of the rotor and make sure to counterbalance the weight*.
7. Spin the device at 14000 x g for 10 minutes. Depending on the Nominal Molecular Weight Limit more time might be required to retrieve all the elute, observe after 10 minutes and repeat if necessary. *Note: The centrifuge column counts with a baseline that assures that the sample does not dry out, therefore some volume will be lost*.

8. Separate the filter from the centrifuge tube.

Label and freeze the filters at -20ºC since they contain treated mucin that could be useful for future experiments.

Negative control: Include a negative control tube with 30 µl of PGM stock solution and 90 µl of reaction buffer.

Periodic acid–Schiff's reagent assay for carbohydrates in a microtiter plate

1. Add into different wells in a micrometer plate:
    a. 25 µl of the eluted sample obtained during centrifugation
    b. 15 µl of the eluted sample obtained during centrifugation
    c. 10 µl of the eluted sample obtained during centrifugation
    d. 5 µl of the eluted sample obtained during centrifugation
    e. 25 µl of the eluted **negative control** obtained during centrifugation
    f. 25 µl of undigested PGM solution (**2.5 µg/µl**) as **positive control**
2. Mix 43 µl of periodic acid stock solution with 677 µl of 7% acetic acid. *Note: Final periodic acid concentration: 0.06% (w/v). For more details see* ***CALCULATIONS***.
3. Add 120 µl of freshly prepared solution (mixed less than 10 minutes before use) of 0.06% (w/v) periodic acid in 7% acetic acid into each well and mix by pipette action.
4. Cover with a plastic seal and incubate for 1.5 hours at 37ºC
5. Allow the mixture to cool down to room temperature, add 100 µl of Schiff's reagent (room temperature) to each well and mix by pipette action
6. Cover with a plastic seal again and shake for 5 minutes.
7. Allow color to develop for 40 minutes at room temperature
8. Remove plastic seal and read absorbance at 550 nm.

CALCULATIONS

Desired final concentration 0.06% (w/v) of periodic acid in 7% acetic acid. Desired final volume 120 µl*6= 720 µl

0.06 %(w/v) = 0.0006 g/ml= 0.6 mg/ml= 0.6 µg/µl

Total mass of periodic acid required: 0.6 µg/µl* 720 µl = **432 µg**

Total volume of 10 µg/µl periodic acid stock solution= 432 µg / 10 µg/µl = **43.2 µl**

REFERENCES

- http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/7/g6920dat.pdf

- Kilcoyne, M., Gerlach, J., Farrell, M., Bhavanandan, V. and Joshi, L. (2011). Periodic acid–Schiff's reagent assay for carbohydrates in a microtiter plate format. *Analytical Biochemistry*, 416(1), pp.18-26.