Alpha- amylase assay

Barley α-amylase is a secreted protein which is normally produced by the aleurone layer to mobilize the starch reserves of the endosperm during seed germination. It is also the major enzyme produced during the malting process. The enzyme is very stable and can be incubated at 60°C during the mashing process in the brewery. For this reason, α-amylase is also used as a tool to measure protein transport through the plant endomembrane system. Tobacco mesophyl protoplasts do not have detectable α-amylase activity, which provides an excellent system for transient expression of this marker molecule.

Solutions required

(Kit from Megazyme Australia)

Substrate : blocked P-nitrophenyl maltoheptaoside (BPNPG7, 54.5 mg)
glucoamylase (100 U at pH 5.2)
α-glucosidase (100 U at pH 5.2)
dissolved in 10 ml water, aliquoted (0.5 ml) and stored at-80°C

Extraction buffer : 50 mM acid malic (sigma M0875)
50 mM sodium chloride
2 µM calcium chloride
0.02 % sodium azide
+ 0.02 % BSA

Stopping buffer : 1 % (w/v) Trizma base

The substrate can be purchased alone, and both Extraction and stopping buffer can be easily made up in the laboratory.

Procedure

Extract cells or dilute culture medium in Extraction buffer according to protoplast harvesting procedures (see other sheets).

Pre-incubate 30 µl of appropriately diluted extract at 45°C in Eppendorf tubes.

Pre-incubate the required amount of substrate (n+1 rule) (in one tube).

After 5 minutes of pre-incubation, start the reaction in 15 second intervals by adding 30 µl of substrate (pipetting up and down 3 times). The reaction can go on for as short as 3 minutes and up to 30 minutes. Stop the reaction in 15 second intervals by adding 150 µl of the stopping buffer. Only then the yellow colour will appear.

Pipet 200 µl into a microtitre plate and read the absorbance at 405nm.

Include controls such as buffer + buffer, buffer + substrate and extract + buffer (the latter only if extract has a colour by itself due to green pigments etc...). Extract + buffer will include any yellow colour that may have emerged due to the instability of the substrate and should be subtracted from the OD values (to give the ΔOD). This is also the reason for the relatively short incubation time (maximum 30 minutes). The difference between extract + buffer and buffer + buffer is the absorbance by plant pigments, which should also be subtracted from the OD value. Usually, this latter value is so small (due to the high dilution of the extracts) that it is not significant.

The ΔOD should be given per ml original cell suspension to allow the calculation of the secretion index. ΔODs above 1.00 are not reliable due to substrate limitation. In that case the assays should be repeated with shorter incubation times or more diluted extracts. It is recommended to do a pilot experiment with one or two samples to determine the ideal dilution prior to analyzing all samples. This can save time and substrate when many extracts are to be analyzed.

It is also possible to calculate the number of units per ml cell suspension, but as we are interested in relative values only, we will not do this at the course.