GlycoSpot – Enzyme screening standard

Sample and plate preparation

- The assays plate (96-well filter plates containing CPH/ICB substrates) are activated by adding 200 µl of activation solution (supplied in the kit) into the wells followed by a 10 minutes incubation at room temperature without agitation
- Vacuum is applied using the vacuum manifold (with spacer block inside and any kind of 96-well plate as a collection plate) or centrifuge (2700xg for 10 min) to remove remaining activation solution
- Wash substrates twice with 100 µl water to remove the stabilizer completely
- Samples with unknown enzyme activity should be diluted/ concentrated to 0.3-3 mg/ml or 30 U/ml, and a signal will be detected after approximately 30min incubation

Assay performance

1. Add 145 µl of an appropriate buffer (the substrates are stable between pH 3.0 to 10.0, CPH-chitosan stable between pH 5-10) including all additional components, e.g. metals or reducing agents needed by the target enzyme
2. Always include buffer alone control, commercial enzymes as positive control and use statistically appropriate number of replicas
3. A 'product plate' is placed underneath the assay plate to collect any potential leakage during incubation
4. The reaction is incubated at an appropriate temperature (the substrates are stable up to 90°C) for a desired time in a shaker at 100-150 rpm agitation. Usually the incubation time is between 30 min and 1 h when testing purified enzymes or 24 h when testing culture broths containing unknown enzyme concentrations
5. The reaction product is transferred into the 'product plate' using a centrifuge (10min at 2700 xg) and can be analyzed further

Detection and Quantification

6. The volume of the reaction product should be equal in each well of the 'product plate'
7. The absorbance is detected at 595 nm for blue substrate (in this case)
8. Calculate the mean absorbance of each sample and control
9. Subtract the value of the negative control from the sample and positive controls
10. Plot the correct absorbance values

Quick start protocol

1. Add 200 µl activation solution to each well in the 96-well plate
2. Incubate 10 minutes at room temperature without agitation
3. Remove the activation solution by centrifugation (2700 xg for 10 min or vacuum)
4. Wash twice with 100 µl water to remove the stabiliser completely
5. Add buffer to each well in the 96-well plate
6. Add samples and controls
7. Put a ‘product plate’ underneath the substrate plate
8. Incubate the reaction at an appropriate temperature with shaking
9. Transfer the reaction product into the product plate by centrifugation(2700 xg for 10 min or vacuum)
10. Check, that the volume in each well is approximately equal
11. Detect the absorbance (404 nm for yellow, 517 nm for red, 595 nm for blue and 630 nm for green substrate)