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4-*O*-Methyl-D-glucurono-D-xylan dyed with Remazol Brilliant Blue R (RBB-Xylan, Remazol Brilliant Blue R D-Xylan)

Product Description:
RBB-Xylan is a soluble chromogenic substrate for the assay of endo-1,4-ß-xylanases.[1]
Prepared by dyeing 4-O-Methyl-D-glucurono-D-xylan with Remazol Brilliant Blue dye. (Dye content approx. 15%)

Solubility / Solution Stability / Substrate Solution:
Suspend 1 g in 100 ml hot distilled water or buffer. Bring to a boil to dissolve. Stir the solution until the powder completely dissolves (about 15 min). Allow to cool to room temperature and adjust the volume to 100 ml. As preservative 200 mg/l sodium azide can be added. With preservative the solution is stable for several years if it is not contaminated with enzyme.

Principle of Assay:
The RBB-Xylan is cleaved with endo-xylanase to low molecular weight dyed fragments which remain in solution on addition of ethanol to the reaction mixture. The high molecular weight fragments are removed by centrifugation and the absorbance of the supernatant is measured at 590 nm. The endo-xylanase activity must be determined by reference to a standard curve.

Precipitation Solution:
For the precipitation an ethanol solution ~96% (v/v) can be used.

Preparing Buffer Solutions:
MES Buffer, 100 mM, pH 6.0

Add 21.3 g of MES (SIGMA 69892) to 900 ml of distilled water and adjust pH to 6.0 with 5 M sodium hydroxide solution (Prod. No. 30530). Adjust the volume to 1 litre.

Sample Preparation:
Pipet 1 ml to 49 ml buffer or dissolve 1 g of sample in 50 ml buffer (this is the stock solution). The powder samples should be gently mixed for about 15 min or until the sample is completely dispersed or dissolved. This solution is clarified by centrifugation (1,500 g, 10 min) or filtration. Most stock solutions will have to be further diluted approximately 100-fold, but the actual dilution will depend on the nature of the sample. After this final dilution, you will have the working sample solution.

Assay:

1. Pipet 0.5 ml of the enzyme sample solution to 0.5 ml substrate solution. Mix and incubate at 40°C for exactly 10 minutes.
2. Terminate the reaction and precipitate the high molecular weight fragments by adding ethanol ~96% (v/v) with vigorous mixing.
3. Allow the tubes to equilibrate to room temperature for 10 minutes, and then mix the tubes again and centrifuge at 1,500g for 10 minutes.
4. The absorbance of the supernatant solution is measured at 590 nm and the enzyme activity is determined by reference to a standard curve.

Calculation of Activity:

1 U corresponds to the amount of enzyme which liberates 1µmol remazol brilliant blue R at pH 6.0 and 40°C.

Determine activity by reference to the standard curve to convert absorbance to milli-Units of activity per 0.5 ml sample solution and then calculate as follows:

Units/ml or g of sample = milli-Units per 0.5 ml sample solution $\cdot \frac{2 \cdot 50 \cdot Dilution}{1000}$

where:

2 = conversion from 0.5 ml to 1.0 ml.

50 = the volume of buffer used to prepare the sample stock solution (i.e. 1.0 g or 1.0 ml of enzyme in 50 ml of buffer).

1000 = conversion from milli-Units to Units.

Dilution = further dilution of the sample stock solution.

References:

1. P. Biely, et al., Soluble chromogenic substrate for the assay of endo-1,4-b-xylanases, *Meth. Enzymol.* **160**, 536 (1988)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.