

Megazyme

ASSAY OF

***endo*-ARABINANASE**

using

**RED
DEBRANCHED
ARABINAN**

S-RDAR 01/14



SUBSTRATE:

The substrate (Red Debranched Arabinan) is prepared from purified, debranched sugar-beet arabinan (Ara: Gal: Rha: GalUA = 77: 11: 5: 7). The arabinan is debranched with α -L-arabinofuranosidase and then dyed with Procion Red dye.

SUBSTRATE PREPARATION:

Add powdered substrate (1 gram) to 45 mL of vigorously stirring water (at room temperature). Stir the suspension and heat (approx. 70°C) until a clear solution is obtained.

Do not overheat as the substrate is not stable to boiling. (High blank values will result if the solution is overheated). Cool the solution to room temperature and add 5 mL of 2 M sodium acetate buffer (pH 4.5). Adjust the pH to 4.5 and the volume to 50 mL. Cool to 4°C and store at this temperature.

ASSAY PROCEDURE:

1. Add 0.5 mL of suitably diluted and pre-equilibrated enzyme solution in 0.1 M sodium acetate buffer (pH 4.5) to pre-equilibrated substrate (0.5 mL), stir the mixture on a vortex mixer and incubate at 40°C.
2. After exactly 10 min, terminate the reaction and precipitate unhydrolysed substrate by the addition of 4.0 mL of laboratory grade ethanol (approx. 95%) with vigorous stirring on a vortex mixer.
3. Allow the tubes to equilibrate to room temperature for 10 min, and then centrifuge on a bench-top centrifuge at 3,000 rpm (1,000 g) for 10 min.
4. Measure the absorbance of the supernatant solution at 520 nm, and calculate the enzyme activity by reference to a standard curve or by using the regression equation.

One Unit of enzyme activity is defined as the amount of enzyme required to release one micromole of arabinose reducing-sugar equivalents (Nelson/Somogyi assay) from CM-Linear Arabinan in one minute at 40°C and pH 4.5.

A typical standard curve for *A. niger* *endo*-arabinase on Red Debranched Arabinan (Lot 110701) is shown below.

To use this curve, the assay conditions described above must be strictly adhered to.

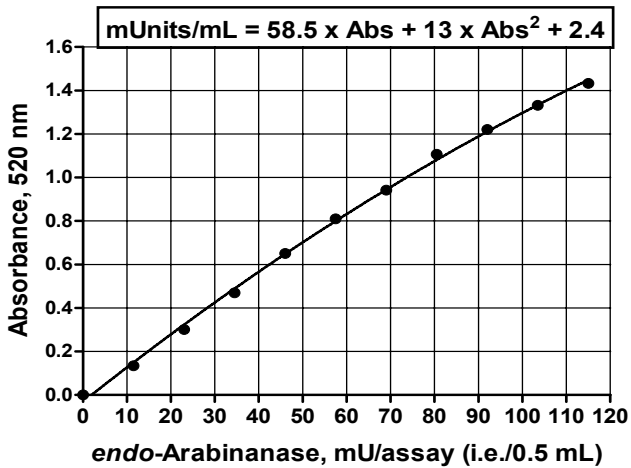


Figure 1. Standard curve for the action of *A.niger* *endo*-arabinase on Red Debranched Arabinan (Lot 110701). Assay conditions are described in the text.

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