

# Megazyme

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ASSAY OF  
*endo*-ARABINANASE  
using

RED  
DEBRANCHED  
ARABINAN

SRDAR 11/99



#### 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 -L-arabinofuranosidase and then dyed with Procion Red dye.

#### SUBSTRATE PREPARATION:

Powdered substrate (1 gram) is added to 45 ml of vigorously stirring water (at room temperature). The suspension is stirred and heated (about 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. Suitably diluted and pre-equilibrated enzyme solution (0.5 ml) in 0.1 M sodium acetate buffer (pH 4.5) is added to pre-equilibrated substrate (0.5 ml) and the mixture is stirred and incubated at 40°C.
2. After exactly 10 min, the reaction is terminated and unhydrolysed substrate is precipitated by the addition of 4.0 ml of laboratory grade ethanol (approx. 95%) with vigorous stirring on a vortex mixer.
3. The tubes are allowed to equilibrate to room temperature for 10 min, and then centrifuged on a bench-top centrifuge at 3,000rpm (1,000 g) for 10 min.
4. The absorbance of the supernatant solution is measured at 520 nm, and the enzyme activity is calculated by reference to a standard curve.

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 90802) is shown below.

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

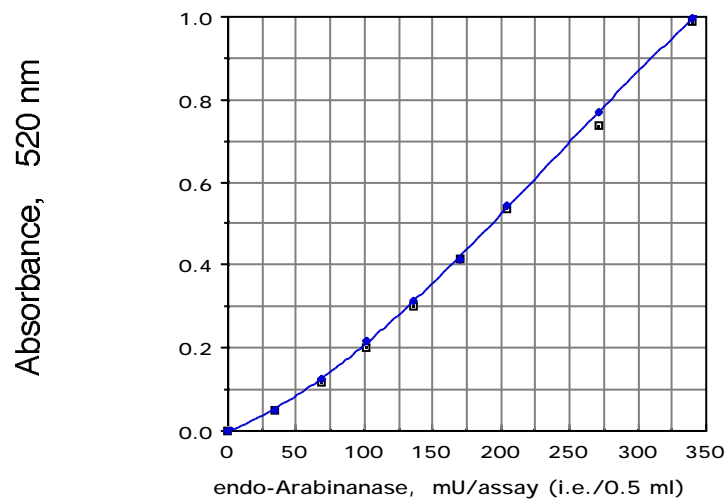


Figure 1. Standard curve for the action of endo-arabinase on Red Debranched Arabinan (Lot 90802). Assay conditions are described in the text.



Megazyme International Ireland Ltd.,  
Bray Business Park, Bray,  
Co. Wicklow,  
IRELAND

Telephone: (353.1) 286 1220  
Facsimile: (353.1) 286 1264  
Internet: [www.megazyme.com](http://www.megazyme.com)  
E-Mail: [info@megazyme.com](mailto:info@megazyme.com)

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