α-L-ARABINOFURANOSIDASE from *Bifidobacterium* sp. (Lot 91201b)

Recombinant

E-AFAM2 **10/12**

(3.2.1.55) alpha-L-arabinofuranoside arabinofuranohydrolase
CAZy: GH Family 43

PROPERTIES

1. ELECTROPHORETIC PURITY:

- Single band on SDS-gel electrophoresis (MW ~ 59,404)
- Broad diffuse band on isoelectric focusing (pI ~ 4.6)

2. SPECIFIC ACTIVITY AND LEVEL OF OTHER ACTIVITIES:

102 U/mg protein (on wheat arabinoxylan) at pH 6.0 and 40°C.

***One Unit** of α-L-arabinofuranosidase activity is defined as the amount of enzyme required to release one µmole of arabinose per minute from wheat arabinoxylan (10 mg/mL) in sodium phosphate buffer (100 mM) pH 6.0.

3. RELATIVE RATES OF HYDROLYSIS OF SUBSTRATES:

Substrate	%
Wheat Arabinoxylan (7 mg/mL; pH 6; 40°C)	100
Xyalanse-treated Wheat Arabinoxylan (7 mg/mL; pH 6; 40°C)	90.4
Sugar Beet Arabinan (7 mg/mL; pH 4; 40°C)	4.0
p-Nitrophenyl-α-arabinofuranoside (2.5 mM; pH 4; 40°C)	0.095

4. OTHER ACTIVITIES (as a percentage of α-L-arabinofuranosidase activity):

Enzyme Activity	%
endo-α-L-Arabinanase	< 0.0001
endo-β-D-Xylanase	< 0.0001

Protein was determined using the Folin/Lowry procedure with BSA as standard.

5. STABILITY:

Stable at room temperature for > 6 h at pH 6.0. Stable for 2 hours at pH 6.0 and temperatures up to 50°C. The enzyme is supplied as an ammonium sulphate suspension in 0.02% (w/v) sodium azide and should be stored at 4°C. Stable for > 2 years at 4°C. On dissolution in buffer, store at -20°C. Stable to repeated freeze-thaw cycles.

6. REFERENCES:

Van Laere, K.M.J., Beldman, G. & Voragen, A.G.J. (1997) A new arabinofuranohydrolase from *Bifidobacterium adolescentis* able to remove arabinofuranosyl residues from double substituted xylose units in arabinoxylan. *Appl Microbiol. Biotech.* **47**: 231-235.

Van den Broek, L.A.M., Lloyd, R.M., Beldman, G., Verdoes, J.C., McCleary, B.V. & Voragen, A.G.J. (2005) Cloning and characterization of arabinoxylan arabinohydrolase D-3 (AXH-D3) from *Bifidobacterium adolescentis* DSM20083. *Appl Microbiol. Biotech.* **67**: 641-647.

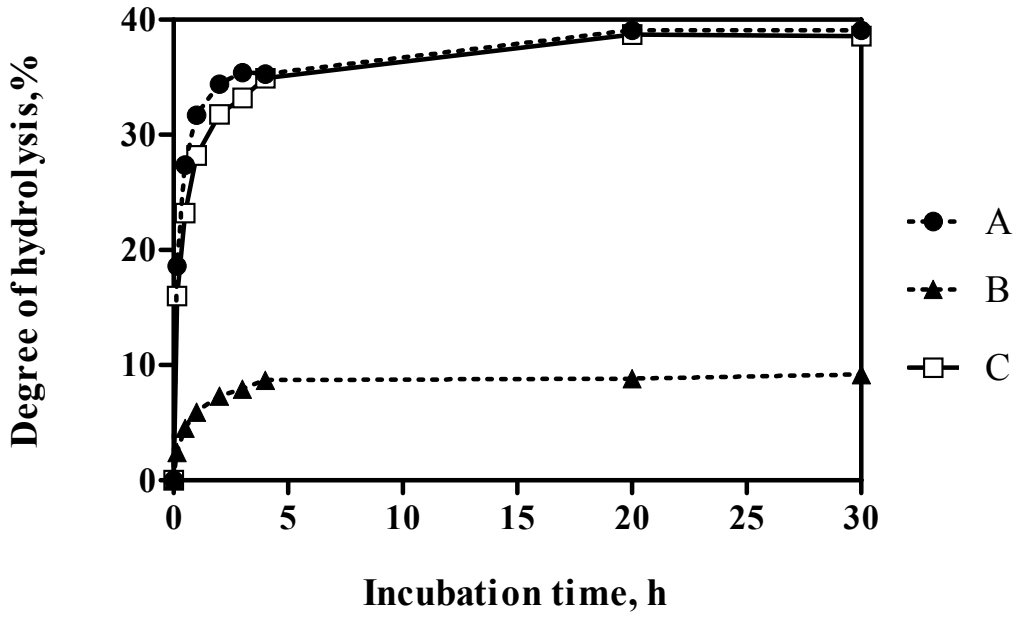

***Figure 1*. Hydrolysis of xylanase degraded wheat arabinoxylan by *A. niger* and *B. adolescentis* α-L-arabinofuranosidase.**

Xylanase degraded wheat arabinoxylan (5 mL, 2 mg/mL) was incubated with A. *A. niger* α-L-arabinofuranosidase (500 U on *p*-NP-α-L arabinofuranoside) in 100 mM sodium acetate buffer (pH 4.5), or B. *B. adolescentis* α-L-arabinofuranosidase (7 U on wheat arabinoxylan) in 100 mM sodium maleate buffer (pH 6.5), or C. *A. niger* α-L-arabinofuranosidase (500 U on *p*-NP-α-L arabinofuranoside) plus *B. adolescentis* α-L-arabino-furanosidase (7 U on wheat arabinoxylan) in 100 mM sodium acetate buffer (pH 5.0). Aliquots (50 mL) were removed at various time intervals, inactivated by incubation at 100°C for 2 min, and analysed for released L-arabinose with β-galactose dehydrogenase. Degree of hydrolysis was calculated as L-arabinose released as a percentage of total carbohydrate determined with the phenol-sulphuric acid procedure.

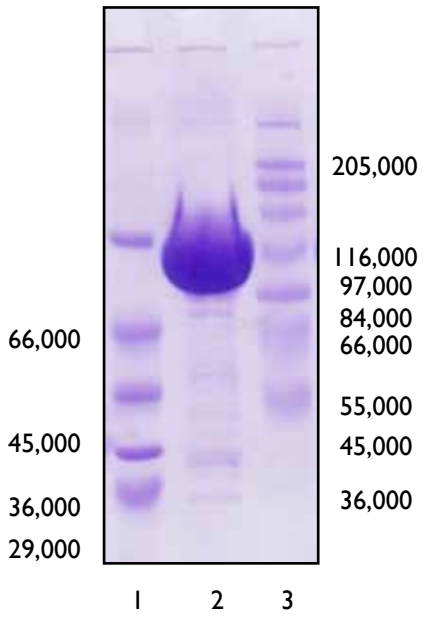

***Figure 2*. SDS-PAGE analysis of α-L-arabinofuranosidase (*Bifidobacter sp.*)**

Electrophoresis was performed using a 10% acrylamide gel.
Lane 1, low molecular weight markers (Sigma cat. no. M-3918); lane 2, 5 µg *B. adolescentis* α-L-arabinofuranosidase; lane 3, high molecular weight markers (Sigma cat. no. M-3788).