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endo-XYLANASE (XylX6 METHOD)

SUPPORTING INFORMATION FOR USE WITH K-XylX6 *endo*-XYLANASE ASSAY KIT

10/16

Relationship between the absorbance obtained using the XylX6 assay with *endo*-1,4-β-xylanase activity on the native substrates, wheat arabinoxylan and beechwood xylan

endo-1,4-β-Xylanases (*endo*-xylanases) from different organisms vary in their ability to hydrolyse different substrates. GH10 Xylanases display much higher activity on unsubstituted β-1,4-xylan and β-1,4-xylo-oligosaccharides than on arabinoxylan due to the highly substituted structure of the latter. Accordingly, GH10 xylanases exhibit higher activity on XylX6 (unsubstituted) followed by beechwood xylan (**P-XYLNBE**) (partially substituted) and wheat arabinoxylan (**P-WAXYM**) (highly substituted) in that order. Broadly speaking, GH11 xylanases do not exhibit large differences in their specific activities across these 3 substrates. The active sites in GH11 xylanases are much better able to accommodate substitution on the xylan backbone. The current document provides for the conversion of the absorbance values obtained using the **XylX6** assay into the relevant activities on the native polysaccharide substrates. A summary of this information is provided in the **XylX6 data booklet**.

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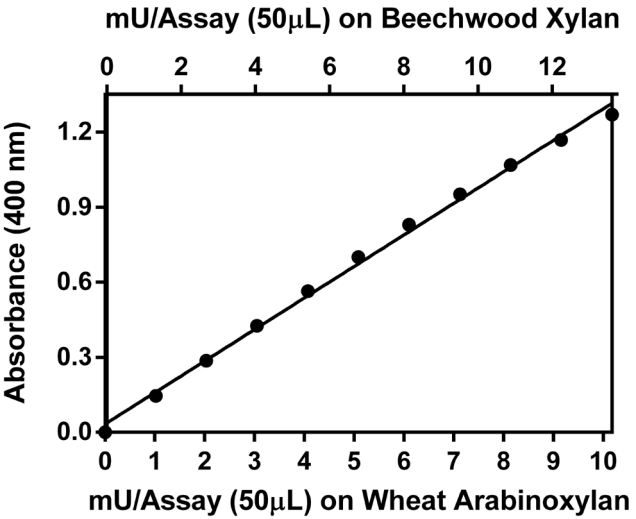

Figure 1. Standard curve relating the activity of *Trichoderma longibrachiatum endo*-1,4-β-xylanase (**E-XYTR3**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Trichoderma longibrachiatum*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 7.904 x ΔA_{400} - 0.239
and **Units on wheat arabinoxylan = 0.87 x XylX6 Units**

mU/Assay on beechwood xylan = 10.681 x ΔA_{400} - 0.324
or **Units on beechwood xylan = 1.17 x XylX6 Units**

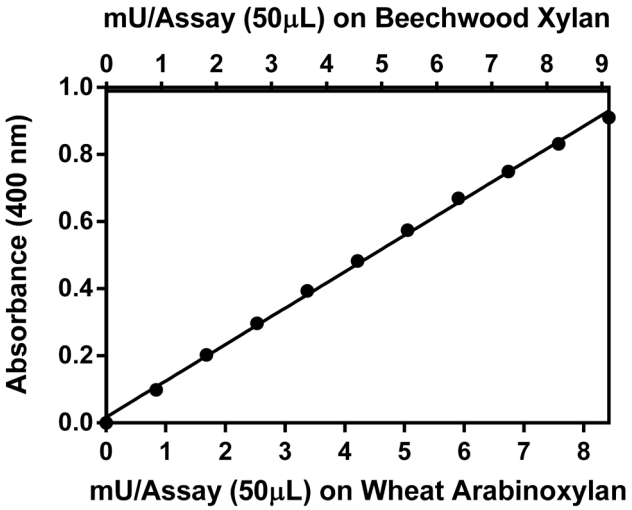

Figure 2. Standard curve relating the activity of *Aspergillus niger endo*-1,4-β-xylanase (**E-XYAN4**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Aspergillus niger*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 9.208 x ΔA_{400} - 0.150
or **Units on wheat arabinoxylan = 1.02 x XylX6 Units**

mU/Assay on beechwood xylan = 9.964 x ΔA_{400} - 0.162
or **Units on beechwood xylan = 1.10 x XylX6 Units**

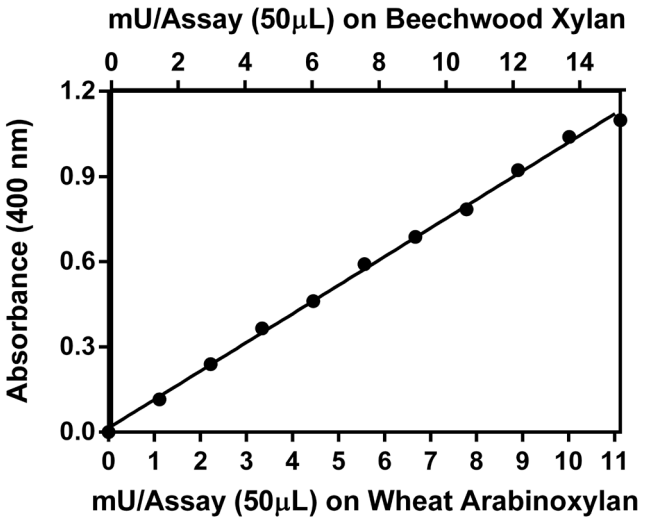

Figure 3. Standard curve relating the activity of *Neocallimastix patriciarum endo*-1,4-β-xylanase (**E-XYLNP**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Neocallimastix patriciarum*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 9.920 x ΔA_{400} - 0.130
or **Units on wheat arabinoxylan = 1.10 x XylX6 Units**

mU/Assay on beechwood xylan = 13.665 x ΔA_{400} - 0.179
or **Units on beechwood xylan = 1.52 x XylX6 Units**

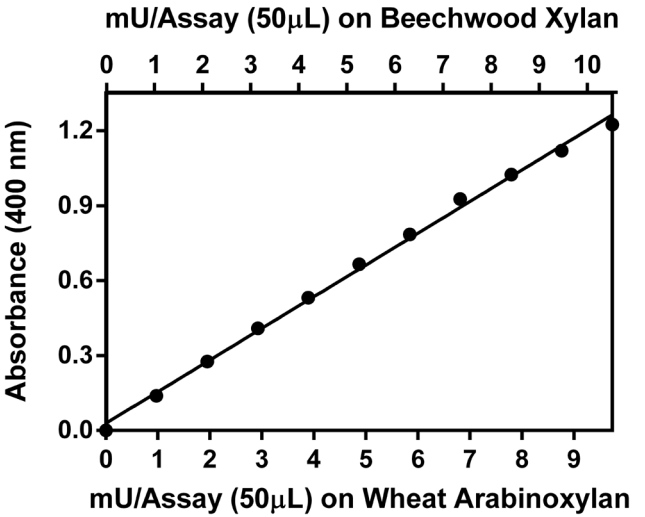

Figure 4. Standard curve relating the activity of *Aspergillus aculeatus* *endo*-1,4-β-xylanase (**E-XYNAA**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Aspergillus aculeatus*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 7.858 x ΔA_{400} - 0.208
or **Units on wheat arabinoxylan = 0.86 x XylX6 Units**

mU/Assay on beechwood xylan = 8.555 x ΔA_{400} - 0.227
or **Units on beechwood xylan = 0.94 x XylX6 Units**

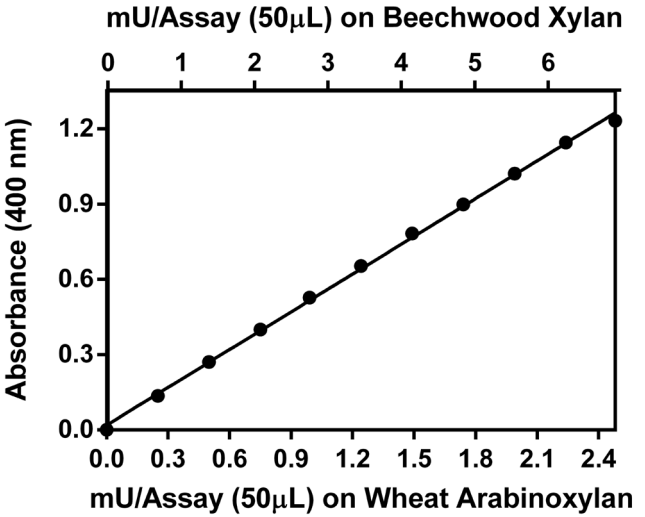

Figure 5. Standard curve relating the activity of *Cellvibrio mixtus endo*-1,4-β-xylanase (**E-XYNBCM**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Cellvibrio mixtus*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 1.993 x ΔA_{400} - 0.038
or **Units on wheat arabinoxylan = 0.24 x XylX6 Units**

mU/Assay on beechwood xylan = 5.590 x ΔA_{400} - 0.105
or **Units on beechwood xylan = 0.66 x XylX6 Units**

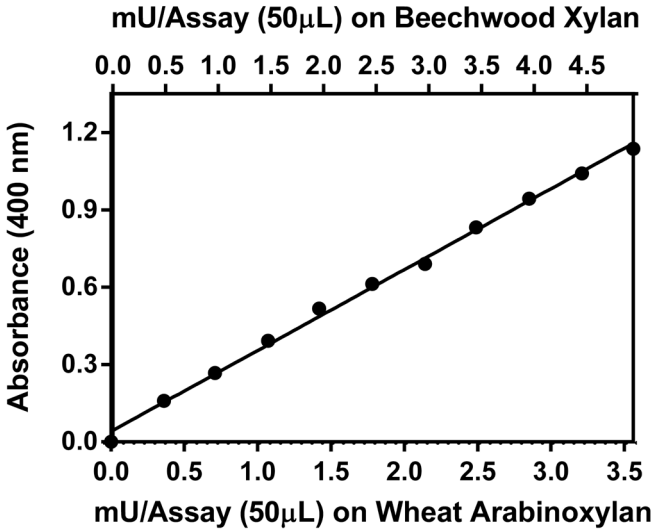

Figure 6. Standard curve relating the activity of *Cellvibrio Japonicus endo*-1,4-β-xylanase (**E-XYNACJ**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Cellvibrio Japonicus*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = = 3.178 x ΔA_{400} - 0.123
or **Units on wheat arabinoxylan = 0.34 x XylX6 Units**

mU/Assay on beechwood xylan = 4.434x ΔA_{400} - 0.172
or **Units on beechwood xylan = 0.48 x XylX6 Units**

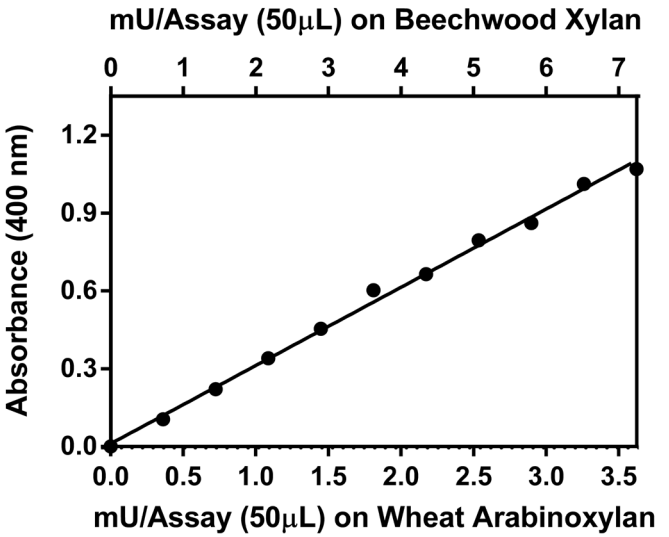

Figure 7. Standard curve relating the activity of *Thermotoga maritima endo*-1,4-β-xylanase (**E-XYLATM**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Thermotoga maritima*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 3.305 x ΔA_{400} - 0.028
or **Units on wheat arabinoxylan = 0.37 x XylX6 Units**

mU/Assay on beechwood xylan = 6.630 x ΔA_{400} - 0.056
or **Units on beechwood xylan = 0.74 x XylX6 Units**

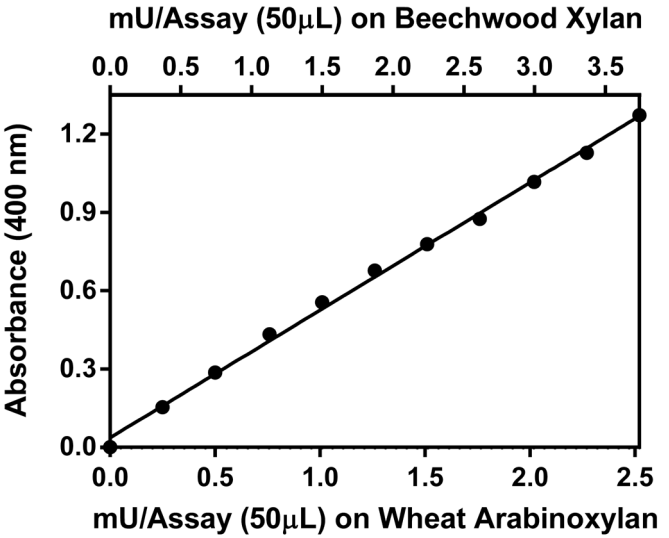

Figure 8. Standard curve relating the activity of *Bacillus stearothermophilus endo*-1,4-β-xylanase (**E-XYNBS**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 400 nm on hydrolysis of XylX6 under the recommended assay conditions.

From this standard curve the following equations can be derived for ***Bacillus stearothermophilus*** *endo*-1,4-β-xylanase:

mU/Assay on wheat arabinoxylan = 2.038 x ΔA_{400} - 0.069
or **Units on wheat arabinoxylan = 0.37 x XylX6 Units**

mU/Assay on beechwood xylan = 3.029 x ΔA_{400} - 0.103
or **Units on beechwood xylan = 0.74 x XylX6 Units**