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Recombinant Enzyme Product Specification Sheet

Cat. No.:	PRO-E0410
LOT:	2009-0410
Activity:	α-Glucosidase
Synonyms:	Maltase; acid maltase; glucoinvertase; glucosidosucrase; lysosomal α-glucosidase; maltase-glucoamylase; α-glucopyranosidase; glucosidoinvertase; α-D-glucosidase; α-glucoside hydrolase; α-1,4-glucosidase; α-D-glucoside glucohydrolase; alpha-glucosidase; alpha-glucopyranosidase; alpha-D-glucosidase; alpha-glucoside hydrolase; alpha-1,4-glucosidase; lysosomal alpha-glucosidase; alpha-D-glucoside glucohydrolase
Nomenclature:	CAZy [GH13 subf21, glycoside hydrolase family 13 subfamily 21, member of clan GH-H], malZ, b0403, JW0393
Source organism:	*Escherichia coli* str. K-12 substr. W3110
Enzyme Commission No.:	3.2.1.20
Activity:	124.3 U/mL (25°C; pH 7.0; 7.69 mg/mL soluble starch)
Specific activity:	34.1 U/mg
Purity:	> 95 % as judged by SDS-PAGE
Form and storage:	Supplied in 3.2 M ammonium sulphate, store at 4°C (shipped at room temperature)
pH optimum:	-
Temperature optimum:	~ 25°C
[Protein]:	3.65 mg/mL
Sequence length:	605 amino acids (view sequence)
Accession No.:	P21517, AP_001053.1, NP_414937.1
Molecular weight:	72992.3 Da (theoretical) ~ 70000 Da (observed by SDS-PAGE) - (observed by mass spectrometry)
Biological function:	Hydrolysis of terminal, non-reducing (1→4)-linked α-D-glucose residues with release of α-D-glucose
Potential application(s):	Carbohydrate research, fundamental research
Comments:	Specificity directed mainly towards the exohydrolysis of 1,4-α-glucosidic linkages

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Usage: Agitate vial sufficiently to fully homogenise enzyme precipitate before use

Assay: One unit is defined as the amount of enzyme required to release 1 µmol of D-glucose equivalents per minute from soluble starch (6.25 mg/mL; Sigma S-9765; ACS reagent; solubilised by boiling for 5 min in H_2O) in 31.25 mM sodium phosphate buffer pH 7.0, containing 0.625 mg/mL BSA, at 25°C, and using the DNSA assay method of Miller (1959; *Anal. Chem.* **31**, 426-428) to follow reducing sugar liberated at 575 nm

NB – for assay 0.1 mL of the enzyme should be centrifuged using a micro-centrifuge at full speed for 2 min to collect the enzyme as an ammonium sulphate pellet. Carefully remove 0.09 mL of the 3.2 M ammonium sulphate supernatant using a yellow tip. The enzyme should then be solubilised by the addition of 0.09 mL of 50 mM sodium phosphate buffer, pH 7.0, containing 1 mg/mL BSA. This enzyme solution (that may be slightly hazy) should then be diluted as necessary for the assay. It is necessary to remove the majority of the ammonium sulphate preservative as described above as this salt interferes with the DNSA assay

A typical assay:

0.50 mL 10 mg/mL soluble starch
0.05 mL 0.5 M sodium phosphate buffer pH 7.0
0.05 mL 10 mg/mL BSA
0.20 mL PRO-E0410 (1/100 dilution)

0.80 mL

Terminate the reaction after incubating at 25°C for 5 min by the addition of 0.75 mL DNSA reagent, followed by boiling for 20 min along with a standard curve (0 - 600 µg D-glucose). As a zero time-point, boil an aliquot of the enzyme dilution for 5 min to inactivate the enzyme. Incubate the zero time-point reaction along with the reaction containing the active enzyme

Primary sequence:

MMLNAWHLPVPPFVKQSKDQLLITLWLTGEDPPQRIMLRTEHDNEEMSVPMHKQRSQPQPGVTAWRAAIDLSSGQ
PRRRYSFKLLWHDRQRWFTPQGFSRMPPARLEQFAVDVPDIGPQWAADQIFYQIFPDRFARSLPREAEQDHVYYH
HAAGQEIILRDWDEPVTAQAGGSTFYGGDLDGISEKLPYLKKLGVTALYLNPVFKAPSVHKYDTEDYRHVDPQFG
GDGALLRLRHNTQQLGMRLVLDGVFNHSGDSHAWFDRHNRGTGGACHNPESPWRDWYSFSDDGTALDWLGYASLP
KLDYQSESLVNEIYRGEDSIVRHWLKAPWNMDGWRLDVVHMLGEAGGARNNMQHVAGITEAAKETQPEAYIVGEH
FGDARQWLQADVEDAAMNYRGFTFPLWGFLANTDISYDPQQIDAQTCMAWMDNYRAGLSHQQQLRMFNQLDSHDT
ARFKTLLGRDIARLPLAVVWLFTWPGVPCIYYGDEVGLDGKNDPFCRKPFPWQVEKQDTALFALYQRMIALRKKS
QALRHGGCQVLYAEDNVVVFVRVLNQQRVLVAINRGEACEVVLPASPFLNAVQWQCKEGHGQLTDGILALPAISA
TVWMN

Literature:
1. Hayashi *et al.* (2006) *Mol. Syst. Biol.* **2**, 1-5
2. Miller (1959) *Anal. Chem.* **31**, 426-428