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Recombinant Enzyme Product Specification Sheet

Cat. No.:	PRO-E0069
LOT:	2009-0069
Activity:	α-Glucuronidase
Synonyms:	α-Glucosiduronase; α-D-glucosiduronate glucuronohydrolase; alpha-glucuronidase; alpha-glucosiduronase; alpha-D-glucosiduronate glucuronohydrolase
Nomenclature:	CAZy [GH67, glycoside hydrolase family 67]
Source organism:	*Cellvibrio japonicus* NCIMB 10462
Enzyme Commission No.:	3.2.1.139
Activity:	183.48 U/mL (37°C; pH 6.5; aldouronic acid mixture – see assay below)
Specific activity:	27.72 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:	6.3
Temperature optimum:	< 55°C
[Protein]:	6.62 mg/mL
Sequence length:	711 amino acids (residues 22-732; view sequence)
Accession No.:	Q8VP74, AAL57752
Molecular weight:	97904.6 Da (theoretical)
	~ 100000 Da (observed by SDS-PAGE)
	- (observed by mass spectrometry)
Biological function:	Catalyses the release of 4-*O*-methyl-D-glucuronic acid from 4-*O*-methyl-D-glucuronoxylooligosaccharides but not from 4-*O*-methyl-D-glucuronoxylan
Potential application(s):	Biomass conversion, carbohydrate research, fundamental research
Comments:	PDB: 1GQI, 1GQJ, 1GQK, 1GQL, 1H41
Usage:	Agitate vial sufficiently to fully homogenise enzyme precipitate before use. When performing DNSA assays, it is necessary to remove the majority of the ammonium sulphate stabilisation solution by

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centrifugation to avoid interference. Re-suspend the resultant enzyme pellet in 50 mM sodium phosphate buffer, pH 6.5, containing 1 mg/mL BSA, prior to assay (under these conditions a very slight haze may be visible. This will not interfere with the assay)

Assay:

One unit is defined as the amount of enzyme required to release 1 µmol of D-glucose equivalents per minute from an aldouronic acid mixture [prepared as follows: 50 mg of 4-*O*-methyl-D-glucurono-D-xylan was dissolved in 3.5 mL of 50 mM sodium phosphate buffer, pH 7.0, containing 1.8 mg/mL *C. mixtus* β-xylanase (cat. no. PRO-E0051). After 60 min incubation with stirring at 37°C, the reaction was boiled for 5 min to inactivate the xylanase. After centrifugation to remove the insoluble xylanase precipitate, the resultant aldouronic acid mixture was used to assay α-glucuronidase activity]. The final assay conditions comprised 2.75 mg/mL aldouronic acid mixture (prepared as described above) in 48.1 mM sodium phosphate buffer, pH 6.5, containing 1 mg/mL BSA, at 37°C, and using the DNSA assay method of Miller (1959; *Anal. Chem.* **31**, 426-428) to follow reducing sugar liberated at 575 nm. **NOTE:** because of a very high blank, this assay **MUST** be performed very carefully, and in triplicate, to ensure accurate results. Alternative assays using purified aldouronic acids, or mixtures thereof, or even borohydride reduced preparations of these substrates, should yield similar results

Primary sequence:

AQTEDGYDMWLRYQPIADQTLLKTYQKQIRHLHVAGDSPTINAAAAELQRGLSGLLNKPIVARDEKLKDYSLVIG
TPDNSPLIASLNLGERLQALGAEGYLLEQTRINKRHVVIVAANSDVGVLYGSFHLLRLIQTQHALEKLSLSSAPR
LQHRVVNHWDNLNRVVERGYAGLSLWDWGSLPNYLAPRYTDYARINASLGINGTVINNVNADPRVLSDQFLQKIA
ALADAFRPYGIKMYLSINFNSPRAFGDVDTADPLDPRVQQWWKTRAQKIYSYIPDFGGFLVKADSEGQPGPQGYG
RDHAEGANMLAAALKPFGGVVFWRAFVYHPDIEDRFRGAYDEFMPLDGKFADNVILQIKNGPIDFQPREPFSALF
AGMSRTNMMMEFQITQEYFGFATHLAYQGPLFEESLKTETHARGEGSTIGNILEGKVFKTRHTGMAGVINPGTDR
NWTGHPFVQSSWYAFGRMAWDHQISAATAADEWLRMTFSNQPAFIEPVKQMMLVSREAGVNYRSPLGLTHLYSQG
DHYGPAPWTDDLPRADWTAVYYHRASKTGIGFNRTKTGSNALAQYPEPIAKAWGDLNSVPEDLILWFHHLSWDHR
MQSGRNLWQELVHKYYQGVEQVRAMQRTWDQQEAYVDAARFAQVKALLQVQEREAVRWRNSCVLYFQSVAGRPIP
ANYEQPEHDLEYYKMLARTTYVPEPWHPASSSRVLK

Literature:

1. Nagy *et al.* (2002) *J. Bacteriol.* **184**, 4925-4929
2. Nagy *et al.* (2003) *J. Biol. Chem.* **278**, 20286-20292
3. Miller (1959) *Anal. Chem.* **31**, 426-428