

***Onchocerca
volvulus* chitinase
(OvCht1)**



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P5206S 003120514051

P5206S



500 units **10,000 U/ml** **Lot: 0031205**
RECOMBINANT **Store at -20°C** **Exp: 5/14**

Description: *Onchocerca volvulus* chitinase (OvCht1) is expressed during development in the black fly vector in the infective L3 larvae and then secreted during post-infective development in the human host (1,2). The specific role of the chitinase is not yet clear.

Source: Isolated from the supernatant of *Spodoptera frugiperda* (Sf9) insect cells infected with an AcNPV chiA minus recombinant baculovirus carrying the OvCht1 gene cloned from *Onchocerca volvulus*.

More Units

AcNPV (*Autographa californica* nuclear polyhedrosis virus)

Supplied in: 200 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM EDTA, 0.1% Triton X-100 and 50% glycerol.

Molecular Weight: The calculated molecular weight of the *Onchocerca volvulus* chitinase enzyme is 53 kDa. Its apparent molecular weight in SDS-PAGE gels is ~65 kDa.

Unit Definition: One unit is defined as the amount of enzyme required to release the equivalent fluorescence produced by 1 pmol of 4-methylumbelliferone from the substrate 4-methylumbelliferyl-N,N',N''-triacetyl-β-chitotrioside in 1 minute at 25°C in a total reaction volume of 100 μl.

Unit Assay Conditions: 0.2 M NaCl, 20 mM NaPO₄ (pH 6.0), 1 mM EDTA, 20 μM 4-methylumbelliferyl-N,N',N''-triacetyl-β-chitotrioside.

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Quality Control Assays

Chitin Binding Assay: 100 units of OvCht1 were added to 100 μl of chitin resin in binding buffer (200 mM NaCl, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA) mixed and left on ice for 15 minutes. After centrifugation to settle the resin, the supernatant was collected containing the unbound protein sample. The resin was then washed twice with 1 ml of binding buffer, centrifuged, and the wash samples collected. The chitinase activity of the initial load, the unbound sample, and the wash samples were determined. Protein samples of the load, unbound protein, wash and resin were also run on an SDS-PAGE gel.

Results: No chitinase activity was detected in the unbound or wash samples. Also, SDS-PAGE gel shows that the 65 kDa band corresponding to the OvCht1 was not present in the unbound or wash samples but was present in the chitin resin sample. Therefore, the OvCht1 was bound to the chitin resin.

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³H-chitin Activity: 160 units of OvCht1 were incubated with 10,000 cpm ³H-chitin in 200 mM NaCl, 20 mM NaPO₄ (pH 6.0), 1 mM EDTA and 500 μg/ml BSA in 200 μl at 37°C. Aliquots were removed at various time points up to 24 hours, mixed with unlabelled chitin, and after centrifugation, the total soluble cpm for each time point was determined.

Results: 160 units of OvCht1 released 59% of the total soluble cpm in 24 hours.

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

References:

1. Wu, Y. et al. (2001) *J. Biol. Chem.* 276, 42557–42564.
2. Wu, Y. et al. (1996) *Mol. Biochem. Parasitol.* 75, 207–219.

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