Brugia malayi chitinase (BmCHT1)

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P5205S 0011205 14051

P5205S

500 units **10,000 U/ml** **Lot: 0011205**

RECOMBINANT **Store at –20°C** **Exp: 5/14**

Description: *Brugia malayi* chitinase (BmCHT1) is expressed in the microfilarial stage, the first larval stage, of the organism and is thought to be important in the exsheathment process of the microfilaria (1). Exsheathment is required for further development of the microfilaria once ingested by the mosquito vector (2). The microfiliaria of *Brugia malayi* have been shown to have chitin in their sheaths (3). Antisera to the chitinase temporarily cleared the microfilaria from the bloodstream of infected jirds (1,4).

Source: Isolated from the supernatant of *Spodoptera frugiperda* (Sf9) insect cells infected with an AcNPV chiA minus recombinant baculovirus carrying the BmCHT1 gene cloned from *Brugia malayi* (kindly provided by J. Fuhrman) (1).

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 *Brugia malayi* chitinase enzyme is 54 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 $NaPO_4$ (pH 6.0), 1 mM EDTA, 20 µM 4-methylumbelliferyl-N,N´,N´´-triacetyl-β-chitotrioside.

Quality Control Assays

Chitin Binding Assay: 100 units of BmCHT1 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 BmCHT1 was not present in the unbound or wash samples but was present in the chitin resin sample. Therefore, the BmCHT1 was bound to the chitin resin.

^{3}H-chitin Activity: 160 units of BmCHT1 were incubated with 10,000 cpm ^{3}H-chitin in 20 mM $NaPO_4$ (pH 6.0), 0.2 M NaCl, 1 mM EDTA, 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 BmCHT1 released 92% 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. Fuhrman, J.A. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 1548–1552.
2. Fuhrman, J.A. (1994) *Exp. Parasitol.* 79, 85–88.
3. Fuhrman, J.A. and Piessens, W. F. (1985) *Mol. Biochem. Parasitol.* 17, 93–104.
4. Canlas, M. et al. (1984) *Am. J. Trop. Med. Hyg.* 33, 420–424.

CERTIFICATE OF ANALYSIS

Brugia malayi chitinase (BmCHT1)

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

P5205S 0011205 14051

P5205S

500 units 10,000 U/ml Lot: 0011205

RECOMBINANT Store at –20°C Exp: 5/14

Description: *Brugia malayi* chitinase (BmCHT1) is expressed in the microfilarial stage, the first larval stage, of the organism and is thought to be important in the exsheathment process of the microfilaria (1). Exsheathment is required for further development of the microfilaria once ingested by the mosquito vector (2). The microfiliaria of *Brugia malayi* have been shown to have chitin in their sheaths (3). Antisera to the chitinase temporarily cleared the microfilaria from the bloodstream of infected jirds (1,4).

Source: Isolated from the supernatant of *Spodoptera frugiperda* (Sf9) insect cells infected with an AcNPV chiA minus recombinant baculovirus carrying the BmCHT1 gene cloned from *Brugia malayi* (kindly provided by J. Fuhrman) (1).

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 *Brugia malayi* chitinase enzyme is 54 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 $NaPO_4$ (pH 6.0), 1 mM EDTA, 20 µM 4-methylumbelliferyl-N,N´,N´´-triacetyl-β-chitotrioside.

Quality Control Assays

Chitin Binding Assay: 100 units of BmCHT1 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 BmCHT1 was not present in the unbound or wash samples but was present in the chitin resin sample. Therefore, the BmCHT1 was bound to the chitin resin.

^{3}H-chitin Activity: 160 units of BmCHT1 were incubated with 10,000 cpm ^{3}H-chitin in 20 mM $NaPO_4$ (pH 6.0), 0.2 M NaCl, 1 mM EDTA, 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 BmCHT1 released 92% 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. Fuhrman, J.A. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89, 1548–1552.
2. Fuhrman, J.A. (1994) *Exp. Parasitol.* 79, 85–88.
3. Fuhrman, J.A. and Piessens, W. F. (1985) *Mol. Biochem. Parasitol.* 17, 93–104.
4. Canlas, M. et al. (1984) *Am. J. Trop. Med. Hyg.* 33, 420–424.

CERTIFICATE OF ANALYSIS