Plasmodium falciparum chitinase (PfCHT1)

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P5208S 003120514051

P5208S

RR Yes

100 units	**2,000 U/ml**	**Lot: 0031205**
RECOMBINANT	**Store at –20°C**	**Exp: 5/14**

Description: *Plasmodium falciparum* chitinase (PfCHT1) is thought to have a role in parasite transmission by acting on the chitin-containing peritrophic membrane of the mosquito vector's bloodmeal to release the ookinetes (1,2). Inhibition of chitinase activity in the mosquito midgut with allosamidin, a chitinase inhibitor, blocks parasitic transmission (3).

Source: Isolated from *E. coli* cells carrying a plasmid with the PfCHT1 gene cloned from *Plasmodium falciparum* (kindly provided by J. Vinetz) (1).

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 *Plasmodium falciparum* chitinase enzyme is 40 kDa. Its apparent molecular weight in SDS-PAGE gels is ~40 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 PfCHT1 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 40 kDa band corresponding to the PfCHT1 was not present in the unbound or wash samples but was present in the chitin resin sample. Therefore, the PfCHT1 was bound to the chitin resin.

^{3}H-chitin Activity: 160 units of PfCHT1 were incubated with 10,000 cpm ^{3}H-chitin in 200 mM NaCl, 20 mM $NaPO_4$ (pH 6.0), 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 PfCHT1 released 30% 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. Vinetz, J.M. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 14061–14066.
2. Tsai, Y. L. et al. (2001) *Infect. Immun.* 69, 4048–4054.
3. Shahabuddin, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 4266–4270.

CERTIFICATE OF ANALYSIS

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