

Recombinant Human Mast Cell Tryptase

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Promega has developed an expression system for generating enzymatically active recombinant human mast cell tryptase^(a) (rhTryptase). Available only from Promega, this enzyme is produced in *Pichia pastoris* and purified by affinity chromatography. Anti-Human Tryptase mAb Biotin also is available from Promega for use in immunostaining, Western analysis and ELISA.

INTRODUCTION

Tryptase, a 135kDa tetrameric serine protease, was originally isolated from lung mast cells (MC). This enzyme is stored in and released from MC granules upon activation. Tryptase is an exquisite marker in heterogeneous populations of MC, allowing for their differentiation from basophils. Because tryptase is the principal protein mediator component of mast cell granules, accounting for 23% of the total cellular protein (1), it is abundant in unstimulated MC and surrounding degranulated MC.

Mast cells are found in many tissues, but are present in greater numbers along the epithelial linings of the body, such as the skin, respiratory tract and gastrointestinal tract. MC are also located in the perivascular tissue surrounding small blood vessels. They are involved in a variety of physiological and pathophysiological states, including immediate hypersensitivity, delayed-type hypersensitivity, cell growth regulation, defense against neoplasia and the sensations of pain and itch (2). MC have also been implicated in chronic inflammatory states and are involved in neuroimmune interactions (3).

Until now, human tryptase has been commercially available only from cadaveric lung tissue. This source has been problematic due to sporadic availability, batch-to-batch variability and concerns about human pathogens contaminating the preparations. The recent availability of rhTryptase, as well as Anti-Human Tryptase mAb Biotin (Cat.# G3361), will aid in research directed toward a more complete understanding of the biological role(s) of tryptase and of mast cells.

RECOMBINANT HUMAN TRYPTASE

Recombinant Human Tryptase (rhTryptase; Cat.# G7061) is expressed as a fully active mature enzyme in *Pichia pastoris* by methanol induction and is purified from the supernatant using affinity chromatography (4). Analysis of the rhTryptase by Coomassie[®]-stained SDS-PAGE illustrates that it is comprised of two major bands with estimated molecular weights of 35.9 and 34.2kDa, a faint band of 33kDa and a diffuse region at approximately 50kDa (Figure 1). The two major bands of protein account for more than 90% of the total reactive protein, as determined by image analysis.

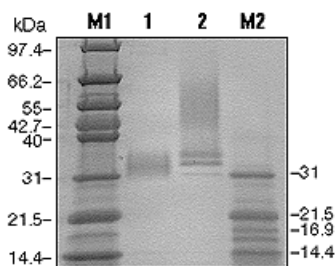


Figure 1. SDS-PAGE analysis of Recombinant Human Tryptase. Purified rhTryptase was analyzed on a 420% reducing Tris-glycine polyacrylamide gel (Novex). Natural human lung tryptase (lane 1) and rhTryptase (lane 2) were loaded onto the gel at 1µg/lane. Proteins were stained with Gelcode (Pierce) and visualized using an AMBIS[™] imaging system (AMBIS, Inc.). Lane M1: Mid-Range Protein Molecular Weight Markers (Cat.# V5231); lane M2: Low-Range Protein Molecular Weight Markers (Cat.# V7551).

Western analysis employing Anti-Human Tryptase mAb Biotin (Cat.# G3361) shows that all of the detectable Coomassie[®]-stained

protein is immunoreactive and therefore, may represent variably glycosylated isoforms (Figure 2). Digestion with peptide N-glycosidase F (PNGase F) and endoglycosidase H (Endo H) confirmed that the heterogeneity of these two isoforms was due to glycosylation since treatment resulted in a single core protein of 33kDa (Figure 2). In comparison, native human tryptase migrates as two major isoforms, along with some minor bands, ranging from 30 to 35kDa. This was reduced to a single band of 29kDa following PNGase F treatment and only partially deglycosylated by Endo H (Figure 2).

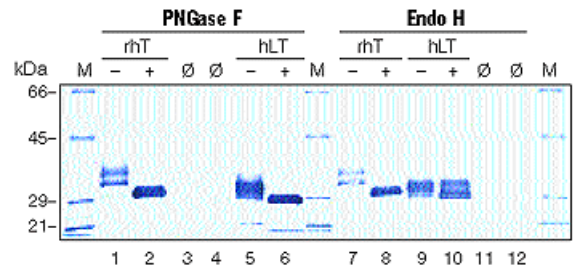


Figure 2. Western blot analysis of rhTryptase and natural human lung tryptase using Anti-Human Tryptase mAb Biotin (Cat.# G3361). Recombinant human tryptase and human lung tryptase were subjected to peptide N-glycosidase F (PNGase F; lanes 1-6) and endoglycosidase H (Endo H, lanes 7-12; both from New England Biolabs) digestions according to the manufacturer's protocol. Briefly, the tryptases (10µg) were denatured at 100°C for 10 minutes in denaturing buffer followed by addition of glycosidases and incubated for 1 hour at 37°C (lane +). Undigested tryptase samples (lane -) were removed prior to the addition of the glycosidases, and controls (lane Ø) included only glycosidases. For Western blot analyses, samples were subjected to SDS-PAGE under reducing conditions and electroblotted onto nitrocellulose, blocked with 1% BSA in 10mM Tris-HCl, 150mM NaCl, 0.05% Tween® 20 (TBST; pH 8.0) and probed with 100ng/ml of Promega's Anti-Human Tryptase mAb Biotin (Cat.# G3361; clone AA5) in TBST. The blots were incubated with a 1:5,000 dilution of Streptavidin Alkaline Phosphatase (Promega Cat.# V5591) in TBST and developed with Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841). Lanes M are broad-range biotinylated molecular weight markers (Bio-Rad). Reproduced with permission from Niles, A.L. *et al.* (1998) *Biotechnology and Applied Biochemistry* **28**, 125. ©Portland Press Limited.

The specific activity of the purified rhTryptase is greater than 1,200U/mg as measured by the Z-Lys-thiobenzyl DTNB-coupled cleavage assay (thiobenzyl/DTNB; Table 1). Similarly, the recombinant enzyme is consistently greater than 35U/mg, measured by hydrolysis of Nalpha benzoyl-DL-Arg-p-nitroanilide (BAPNA) and has a TGPL (tosyl Gly-Pro-Lys-p-nitroanilide) value >300U/mg (Table 1). When compared to fully active native human tryptase, the specific activity of rhTryptase was greater in both enzymatic assay systems (Table 1).

Table 1. Comparison of Specific Activity of rhTryptase to Native Tryptase.

Assay Method	rhTryptase (Units/mg)	Native Tryptase (Units/mg)	Percent Activity of Native Protein
BAPNA	42.0	25.2	166
TGPL	34.5	21.0	164
Thiobenzyl/DTNB	1,773	1,374	129

Native tryptase was derived from human cadavers.
 BAPNA, Nalpha benzoyl-DL-Arg-p-nitroanilide.
 TGPL, tosyl Gly-Pro-Lys-p-nitroanilide.

ANTI-HUMAN TRYPTASE MAB BIOTIN

Immunodetection has improved identification of tryptase and mast cells over previous detection methods such as metachromatic staining. Specific monoclonal antibodies offer a uniform, sensitive and reproducible reagent for the development of enzymatic assays for tryptase. Promega's Anti-Human Tryptase mAb Biotin is derived from hybridoma clone AA5 (5), and has been successfully employed in a variety of applications. Figure 2 illustrates its use in Western analysis (1:10,000 dilution). When matched with an appropriate capture reagent for a sandwich ELISA or by itself in a direct ELISA, the Anti-Human Tryptase mAb Biotin also makes an excellent detection antibody (1:2,000 dilution; data not shown). Furthermore, this antibody is effective for immunolabeling (1:1,000 dilution) MC in a variety of tissue types, using different fixatives (Figure 3).

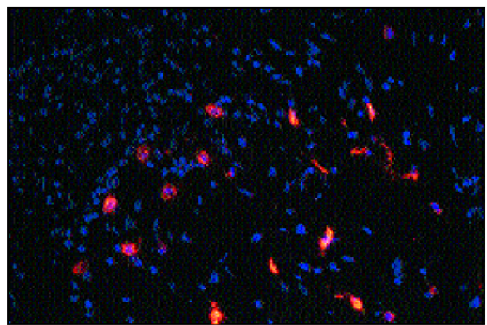


Figure 3. Immunostaining reveals mast cells in bladder tissue. Human bladder from a bladder cancer patient, immunostained with Anti-Human Tryptase mAb Biotin (Cat.# G3361). Note the presence of both intact and degranulated mast cells. Streptavidin Cy3™ (Jackson ImmunoResearch Laboratories, Inc.) was used for detection (visualized through a rhodamine/DAPI filter). Nuclei (blue) were stained with DAPI.

SUMMARY

Recombinant Human Tryptase and Anti-Human Tryptase mAb Biotin have been tested in a variety of assay systems. The rhTryptase has been shown to have a higher specific activity in enzymatic assays than native tryptase. In addition, Anti-Human Tryptase mAb Biotin is useful for a variety of applications, from Western analysis to immunostaining. The uniformity of the recombinant enzyme and hybridoma-derived antibody provides a superior, robust and pathogen-free source of protein for enhancing research on tryptase and mast cells.

REFERENCES

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Ordering Information

Product	Size	Cat.#
rhTryptase	100µg	G7061
Anti-Human Tryptase mAb Biotin	20µg	G3361

Please contact Promega for information on Bulk Purchases of rhTryptase.

Related Products

Product	Size	Cat.#
Streptavidin Alkaline Phosphatase	0.5ml	V5591
Western Blue® Stabilized Substrate for Alkaline Phosphatase	100ml	S3841

^(a)Patent Pending.

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