

Recombinant Human Thyroid Peroxidase (rTPO)

Thyroid Peroxidase (TPO) is an integral apical membrane glycoprotein of thyroid follicular cells, which is responsible for the tyrosine residues in thyroglobulin, leading to thyroid hormone generation (Ruf and Carayon, 2006). In membrane TPO is found as a homodimer with subunits of approximately 100 kDa molecular weight (Baker et al. 1994). The anti-thyroid peroxidase autoantibodies are the most frequently represented autoantibodies in the sera of patients suffering from autoimmune thyroid disease; they are present in 90% of Hashimoto's thyroiditis and 75% of Graves' disease patients (Mariotti et al. 1990). Thus immunoassays for quantification of anti-TPO autoantibodies are widely used in clinical practice.

For many years human native TPO, purified from thyroid glands, has been used as a key component - an antigen - in such assays. Studies in early 90s revealed that soluble extracellular domain of recombinant human thyroid peroxidase (rTPO) produced in insect cells has immunochemical properties similar to native human thyroid peroxidase (Haubruck et al. 1993). The technical limitations in the production of bulk amounts of rTPO have imposed some restrictions on the wide rTPO utilization in assays.

Recombinant human TPO offered by Hytest has immunochemical properties similar to the native antigen purified from human thyroid glands. It can be utilized as an antigen in the assays for the detection of human TPO-specific autoantibodies in the blood of the patients with autoimmune thyroid diseases.

Hytest offers recombinant human TPO expressed in insect cells as soluble extracellular domain of human TPO (amino acid residues 19-846). Calculated molecular weight of the protein 91981 Da. Recombinant human TPO does not contain any tags. The protein is purified to homogeneity using specific monoclonal antibody-

based affinity and ion exchange chromatography methods (Fig. 1). Two visible protein bands (in the region of 100-110 kDa) on the gel after SDS-PAGE gel electrophoresis belong to TPO since both react with specific monoclonal antibodies in Western blotting. The electrophoretic heterogeneity of TPO is apparently caused by differential glycosylation of the protein. The identity of TPO was also confirmed by MALDI mass-spectrometry.

Immunochemical properties of human rTPO were analyzed in comparison with TPO purified from human thyroid glands (native human TPO). Sera of patients with various autoimmune thyroid diseases were tested in ELISA with both TPO preparations used as an antigen for plate coating (Fig. 2).

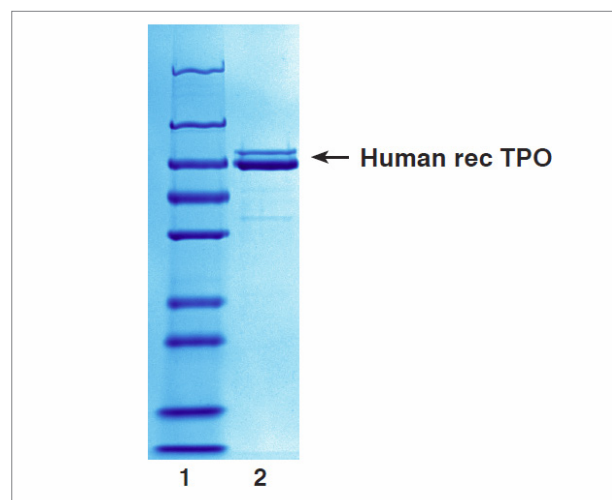


Figure 1. SDS-PAGE of human recombinant TPO expressed in insect cells, reducing conditions.

Lane 1: Molecular weight standards, Fermentas (250, 130, 100, 70, 55, 35, 25, 15, and 10 kDa)

Lane 2: Human recombinant TPO, 1 µg.

Gel staining: Coomassie brilliant blue G-250.

As it follows from Fig. 2, recombinant human TPO produced by Hytest has immunoreactivity very similar to that of the native. Correlation coefficient between the immunochemical activity values obtained for recombinant and native TPO was 0.92 (n=28).

These data suggest that Hytest's recombinant human TPO can be successfully used as an antigen in the assays for the detection of human TPO-specific autoantibodies.

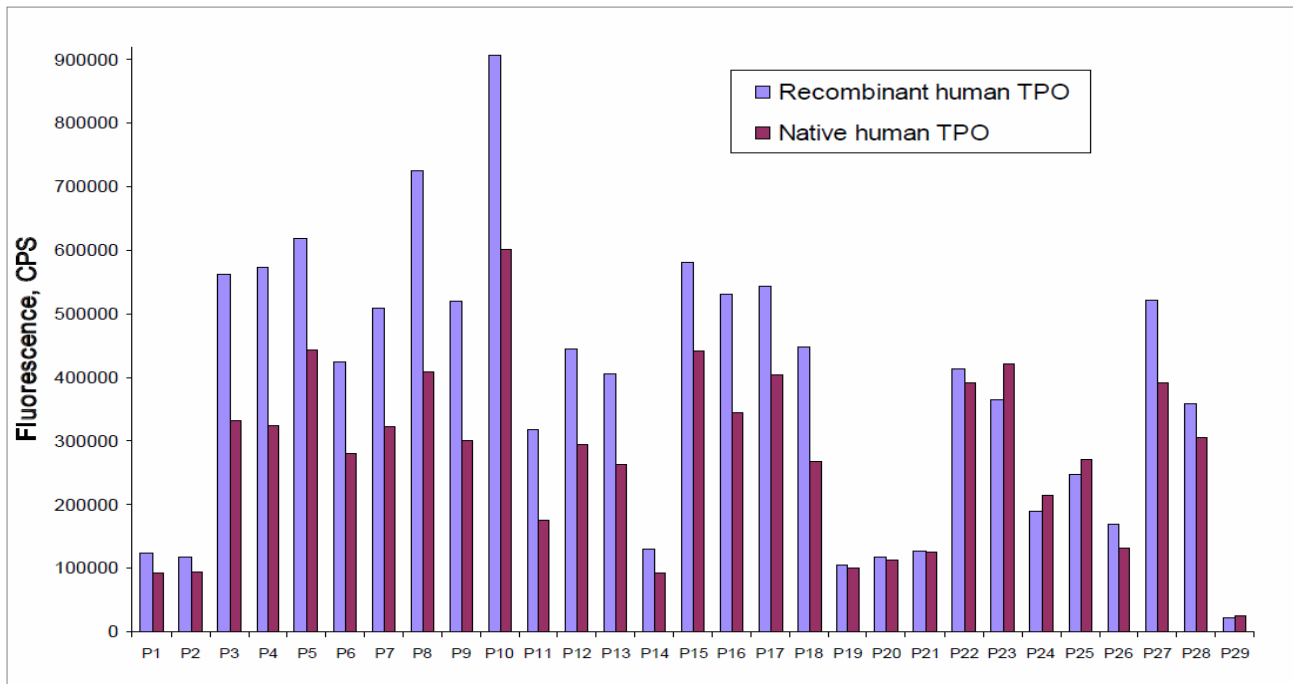


Figure 2.

Comparison of immunochemical properties of recombinant human TPO and native human TPO used as antigens for plate coating in ELISA. Immunoassay plates were coated with rTPO or native TPO (0.1 µg/well). Sera of 28 patients with autoimmune thyroid diseases as well as one healthy patient (P29) were diluted 1/50 and incubated in wells for 1h. Then bound autoantibodies were detected with anti-human IgG antibodies labeled with stable Eu³⁺ chelate. Eu³⁺-fluorescent signals are expressed in CPS.

REFERENCES

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Thyroid peroxidase (TPO)	4TP15	6H7	IgG1	EIA
		TPO28	IgG1	EIA WB
		TPO34	IgG1	EIA, WB
		TPO35	IgG1	EIA

ANTIGEN

Product name	Cat. #	Purity	Source
Thyroid peroxidase (TPO), recombinant	8RTPO	>95%	Recombinant