R&D Ab ®
Research & Diagnostic Antibodies
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IgG Fraction of Rabbit Anti-Human Thrombopoietin Sera

AS-4355G Lot # 9548

These pooled antisera were raised in rabbits which were immunized with a peptide analogue of human thrombopoietin (TPO residues #288-315) attached onto a carrier protein. The antisera have been shown to be specific for TPO, have been characterized by western blotting, ELISA, and cell staining techniques, and are suitable for immunocytochemical and western immunoblotting detection of this hormone. Dilute the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antibody.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Thrombopoietin Human (288-315)	100
Thrombopoietin Human recombinant	~90
Erythropoietin Human recombinant	0

Immunocytochemical Staining

The pooled antisera have been found to stain fixed cells know to express human TPO using ABC techniques at a 1:500 dilution.

Western Immunoblot

Western immunoblots using tissue homogenates have been successful at a 1:800 dilution and yield a single band at 59 kDa.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the antibody after preparing a 1:800 dilution (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash for 5 minutes x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.