

IgG Fraction of Rabbit Anti-Human Thrombopoietin Sera

AS-4354G

Lot # 9541

This IgG fraction was prepared by precipitation, dialysis and chromatography from a pool of antisera that were raised in rabbits which were immunized with a peptide analogue of human thrombopoietin (TPO residues #244-277) attached onto a carrier protein. The whole antisera have been shown to be specific for TPO and have been characterized by western blotting, ELISA, and cell staining techniques. This IgG fraction is suitable for immunocytochemical and western immunoblotting detection of this hormone. Dilute the lyophilized IgG fraction with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent concentration of IgG in whole antiserum, or with additional buffer for more dilute antibody. Each vial contains 2.24 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Thrombopoietin Human (244-277)	100
Thrombopoietin Human recombinant	~90
Erythropoietin Human recombinant	0

Immunocytochemical Staining

The purified IgG fraction has been found to stain fixed cells known to express human TPO using ABC techniques at a concentration of 40 µg/ml.

Western Immunoblot

Western immunoblots using tissue homogenates have been successful at a concentration of 20 µg/ml 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 as 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the IgG fraction after diluting to 20 µg/ml (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.