

**Anti-human Thrombopoietin (TPO)  
Monoclonal Antibody Clone 10A10-D1**Supplied as an IgG Fraction from Ascites Fluid  
MC-4353 Lot # 9567

This purified IgG fraction was isolated from ascites fluid containing mouse monoclonal antibody 10A10-D1 raised against recombinant human thrombopoietin (hTPO). The 50 µg of IgG has been packaged in 0.10 ml of 10 mg/ml BSA in PBS as carrier protein. This monoclonal antibody has been found to bind specifically to residues #244 - 277 of hTPO by indirect ELISA and by western blotting. This monoclonal antibody was tested for recognition of EPO, insulin and other hormones by ELISA techniques. It has been found to be a mouse IgG2b kappa by isotyping.

**Monoclonal Antibody Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
recombinant TPO (human)	100
TPO (244-277)	100
recombinant EPO (human)	0
ACTH (1-39)	0
Calcitonin (human)	0
Insulin (human)	0
Insulin-like Growth Factor 1 (human)	0
Insulin-like Growth Factor 2 (human)	0
Parathyroid Hormone (human)	0

**Indirect ELISA**

The monoclonal antibody in this IgG fraction has been found by indirect ELISA to bind to recombinant hTPO and hTPO(244-277). Titration experiments by indirect ELISA show that 50 ng of this IgG will yield an OD of greater than 2.0 under standard assay conditions.

**Western Immunoblot**

Western immunoblots resulted in a single band being detected at ~ 46 kDa at a protein concentration of 200 ng/ml.

**Western Blotting Protocol**

1. After SDS-PAGE on 12% gels and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the IgG fraction after diluting to at least 200 ng/ml (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2 - 4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-mouse IgG antiserum diluted 1:2500 (Note: dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20 buffer. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 min. per wash cycle in a fairly large volume of TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.