

**Anti-human Thrombopoietin (TPO)
Monoclonal Antibody Clone 10A10-D1**

Supplied as Ascites Fluid (sterile filtered and packaged)
MC-4352 Lot # 9566

This ascites fluid contains mouse monoclonal antibody 10A10-D1 raised against recombinant human thrombopoietin (hTPO). This monoclonal antibody has been found to bind specifically to residues #244 - 277 of hTPO by indirect ELISA and by western blotting techniques. 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. Each vial contains 0.10 ml of sterile filtered ascites fluid.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
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

This monoclonal antibody containing ascites fluid has been found by indirect ELISA to bind to recombinant hTPO and hTPO(244-277). Titration experiments show the titer to greater than 1:4000 by indirect ELISA.

Western Immunoblot

Western blots resulted in a single band being detected at ~ 46 kDa at a dilution of 1:1000.

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 in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the ascites after dilution at least 1:1000. (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.