

**Anti-human Leptin (131-145) Monoclonal Antibody
Clone 8F7-A10**

Supplied as Ascites Fluid (sterile filtered and sterile packaged)
MC-5352 Lot # 9384

This ascites fluid contains anti-human leptin monoclonal antibody clone 8F7-A10 raised against synthetic human leptin (131-145) attached to a carrier protein. This monoclonal antibody has been shown to bind to the carboxyl terminal region of the intact protein. This monoclonal antibody has been shown to bind to intact leptin specifically in ELISAs, in western immunoblots and by immunocytochemistry. It has been found to be mouse IgG_{2A} by isotyping. The ascites fluid has been sterile filtered and sterile packaged.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
Leptin (Human)	100
Leptin (131-145)	100
Epidermal Growth Factor (Human)	0
Insulin (Human)	0
Insulin-like Growth Factor 1 (Human)	0
Insulin-like Growth Factor 2 (Rat)	0
Parathyroid hormone (Human)	0
Transforming Growth Factor-alpha (Human)	0

Immunofluorescent Staining of Cells

This ascites fluid has been found to stain specifically human adipocytes at a 1:1000 dilution. The ability of this monoclonal antibody to bind to leptin in adipocytes was examined in cells fixed with neutral buffered formalin. The fixed cells were incubated for 20 min with 4% normal goat serum, reacted for 60 minutes with the diluted mouse monoclonal antibody, and then with FITC-conjugated goat anti-mouse IgG. The immunofluorescent staining pattern was observed using epifluorescence microscopy.

Western Immunoblot

Western immunoblots of adipocyte homogenates resulted in a single band being detected at ~ 16 kDa at a dilution of 1:4000.

Western Blotting Protocol

1. After SDS-PAGE on 12% percent gels and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the ascites fluid after preparing a 1:4000 dilution. 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-mouse 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 min x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.