R&D ® Ab
Research & Diagnostic Antibodies
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Anti-human Leptin (131-145) Monoclonal Antibody Clone 8F7-A10

Supplied as the IgG Fraction from Ascites Fluid
MC-5353 Lot # 9385

This IgG fraction was isolated from ascites fluid and contains mouse monoclonal antibody 8F7-A10 raised against synthetic human leptin (131-145) attached to a carrier protein. The 50 μgm of purified IgG fraction has been packaged in 0.10 ml of 10mg/ml BSA in PBS as carrier protein. This monoclonal antibody has been shown to bind to the carboxyl terminal region of the protein and has been found 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.

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 monoclonal antibody has been found to stain specifically human adipocytes at a concentration of 0.50 μg/ml. 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 epifluorescense microscopy.

Western Immunoblot

Western immunoblots of adipocyte homogenates resulted in a single band being detected at ~ 16 kDa at a concentration of 0.25 μg/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 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 IgG fraction after preparing a 0.25 μg/ml solution. Use 2% normal goat serum in TBS/Tween-20 as buffer. 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.