R&D Ab
Research & Diagnostic Antibodies
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IgG Fraction of Rabbit Anti-Leptin Serum

AS-5351G Lot # 9289

This IgG fraction was prepared by precipitation, dialysis and chromatography from an antiserum that was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of Leptin covalently attached onto a carrier protein. The antiserum has been shown to be specific for the COOH terminal of Leptin and has been characterized by western blotting, ELISA, and cell staining techniques. The IgG fraction is suitable for immunocytochemical and western immunoblotting detection of intact leptin. Dilute the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent concentration in whole antiserum, or with additional buffer for more dilute antibody. Each vial contains 1.47 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Leptin (Human)	100
Leptin (Rat)	~75
Leptin (Mouse)	~75
Epidermal growth factor (Human)	0
Insulin (Human)	0
Insulin-like Growth Factor 1 (Human)	0
Insulin-like Growth Factor 2 (Human)	0
Transforming growth factor-alpha (Human)	0

Immunocytochemical Staining

This IgG fraction has been found to stain specifically human adipocytes at a concentration of 20 μg/ml. The ability of this rabbit IgG to bind to leptin in adipocytes was examined in cells fixed with neutral buffered formalin. The fixed cells were incubated for 20 minutes with 4% goat serum, reacted for 60 minutes with the diluted rabbit antibody, and then with FITC-conjugated goat anti-rabbit IgG. The immunofluorescent staining pattern was observed using epifluorescence microscopy.

Western Immunoblot

Western immunoblots using adipocyte homogenates have been successful at a concentration of 20 μg per ml and yield a single band at ~ 16 kDa.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 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 dilution to 20 μg per ml (Note: higher dilutions may be needed). Use 2% normal goat serum in h 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.