

Rabbit Anti-Human Leptin[1-24] Serum

AS-5361S

Lot # 9290

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the amino terminal of human Leptin[1-24] covalently attached onto a carrier protein. This antiserum is specific for the amino terminal of human Leptin, is suitable for immunocytochemical and western immunoblotting, and cross reacts with the amino terminal of rat leptin. The antiserum has been characterized by western blotting, ELISA, and cell staining techniques. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Leptin (Human)	100
Leptin (1-24)	100
Leptin (Rat)	~90
Epidermal Growth Factor (Human)	0
Insulin (Human)	0
Insulin-like Growth Factor 1 (Human)	0
Insulin-like Growth Factor 2 (Rat)	0
Transforming Growth Factor-alpha (Human)	0

Immunocytochemistry

This polyclonal rabbit antibody has been found to stain specifically human adipocytes at a 1:500 dilution. The ability of this rabbit antibody to bind to leptin in adipocytes was examined in cells fixed in neutral buffered formalin. The fixed cells were incubated for 20 min with 4% normal 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 result in a single band at ~16 kDa at a dilution of 1:1000.

Western Blotting Protocol

1. After SDS-PAGE on 15% 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 rabbit antibody after preparing a 1:1000 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-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 min x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.