Rabbit Anti-Leptin Sera

AS-5351S   Lot # 9288

The pooled antisera were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of human leptin covalently attached onto a carrier protein. The pooled antisera are specific for the COOH terminal of leptin and are suitable for immunocytochemistry and western immunoblotting. The pooled antisera have been characterized by western blotting, ELISA, RIA and cell staining techniques. Dilute the lyophilized antisera with 0.1 ml of 10 mg/ml BSA is PBS for the equivalent of whole pooled antisera, or with additional buffer for more dilute antisera.

Antisera 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 (Rat)	0
Transforming growth factor-alpha (Human)	0

Immunocytochemical Staining

The antisera have been found to stain fixed adipocytes known to express leptin using ABC techniques at a dilution of 1:800.
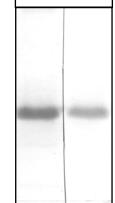
Western Immunoblot

Western immunoblots using adipocyte homogenates have been successful at a dilution of 1:500 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 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:500 (Note: higher dilutions may be needed). 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 minutes x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.

Lane 1 = rhLeptin
Lane 2 = adipocyte homogenate