

**Western Immunoblotting Reagents:  
IgG Fraction of Rabbit Anti-Leptin Serum**

WR-5361P

Lot # 9334

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the amino terminal of Leptin covalently attached onto a carrier protein. The IgG fraction of the rabbit antiserum was prepared by precipitation, dialysis, and column chromatography. Rehydrate the lyophilized IgG fraction with 5.0 ml of TBS/Tween 20 that contains 1% normal goat serum (NGS). The stock solution should be further diluted 1:8 with additional buffer prior to use (see below). This should be sufficient for at least 20 lanes. This antiserum has been found to stain specifically Leptin in western immunoblots.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Leptin (Human)	100
Leptin (1-24)	100
Leptin (Rat)	~90
Epidermal growth factor (Human)	0
Insulin-like Growth Factor 1(Human)	0
Insulin-like Growth Factor 2 (Rat)	0

**Western Blotting Protocol**

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls dissolve 150 nmole of peptide PS-5361 in 600 µl of reconstituted stock antibody. Incubate one hour. Then add 5.4 ml of 1% normal goat serum in TBS-Tween and use 2.0 ml per lane this should be sufficient for 3 blocked control lanes. **DO NOT ADD THE PEPTIDE TO THE STOCK POLYCLONAL ANTIBODY. THIS WILL BLOCK ALL BINDING.**
4. Apply the rabbit polyclonal antibody after dilution to at least 1:8 (Note: higher dilutions may be needed). Use 1% normal goat serum in TBS/Tween 20 with 1% NGS as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 1% normal goat serum in TBS/Tween-20 . Incubate 1-2 hours.
7. Wash x 4 for 5 minutes per wash cycle of TBS/Tween-20.
8. Develop color using the enhanced DAB reaction.

**PS-5361: Leptin amino terminal peptide**

Amino Acid Sequence:

NH<sub>2</sub>-Val-Pro-Ile-Gln-Lys-Val-Gln-Asp-Asp-Thr-Lys-Thr-Leu-Ile-Lys-Thr-Ile-Val-Thr-Arg-Ile-Asn-Asp-Ile-Ser-Cys-COOH

Mol. Wt.: 2941.49

Peptide Quantity: 150 nmoles

Peptide Lot# 9398