R&D ® Ab

Research & Diagnostic Antibodies

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Western Immunoblotting Reagents:
IgG Fraction of Anti-Leptin Monoclonal Antibody 8F7-A10

WR-5351M Lot # 9331

The IgG fraction was purified from ascites fluid and contains mouse monoclonal antibody 8F7-A10 raised against recombinant Leptin. This monoclonal antibody has been shown to bind to intact leptin by ELISA, immunocytochemistry, and western blot analyses, and it has been shown to bind specifically to the carboxyl terminal region of leptin, residues #131-145. The lyophilized powder should be dissolved in 5.0 ml of Tris buffered saline (TBS) that contains 1.0 mg/ml BSA and aliquots should be diluted an additional 1:8 prior to use (See below). This should be sufficient for at least 20 lanes. The 8F7-A10 monoclonal antibody has been shown to be specific for leptin by ELISA and western blot techniques.

Antibody Specificity

Polypeptide/Protein	% 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

Western Blotting Protocol

Western immunoblots resulted in a single band being detected at ~16 kD.

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 2% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. For blocked antibody controls, dissolve 150 nmoles of PS-5351 in 600 µl of stock antibody solution. Incubate one hour. Then add 5.4 ml of 1% normal goat serum in TBS/Tween-20 buffer and use 2.0 ml per lane. This should be sufficient for 3 blocked antibody control lanes. DO NOT ADD THE PEPTIDE TO THE STOCK MONOCLONAL ANTIBODY. THIS WILL BLOCK ALL BINDING.
4. Apply the IgG fraction of the monoclonal antibody after preparing a further 1:8 dilution of the stock solution (Note: higher dilutions may be needed depending upon the detection system used). Use 1% normal goat serum in TBS/Tween-20 as buffer and let the primary antibody bind for 2-4 hours.
5. Wash x 3 with TBS/Tween-20.
6. Apply affinity purified HPR-goat anti-mouse IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 1% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
7. Wash x 4 for 5 min per wash in TBS/Tween-20.
8. Develop color using enhanced DAB reaction.

PS-5351: Leptin [131-145]

Amino Acid Sequence:

NH_2-Cys-Ser-Leu-Gln-Asp-Met-Leu-Trp-Gln-Leu-Asp-Leu-Ser-Pro-Gln-Ser-COOH

Mol. Wt.: 1863.13
Peptide Purity: 95%
Peptide Quantity: 150 nmoles
Peptide Lot # 8943
HPLC Analysis:

Solvent System: A. 0.05 M KH_2PO_4, pH 3.0
B. 70% AcCn + 30% A

Solvent Program:

Time	Flow	%A	%B
0	1.2	100	0
30	1.2	25	75
31	1.2	0	100
32	1.2	100	0
35	1.2	100	0

Detection: optical density at 225 nm
Results: single peak at R_t = 26.100 min