

Product Specification Sheet

Phospho OBRb (Tyr985) Antibodies

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| Cat # OBR14-P | Mouse OBRb Phospho (Y985) peptide # 1 | SIZE: 100 ug |
| Cat # OBR15-P | Mouse OBRb non-phospho peptide # 2 | SIZE: 100 ug |
| Cat # OBR14-A | Rabbit Anti-Mouse phospho-OBRb (Y985) IgG # 1, aff. pure | SIZE: 100 ug |

Obesity, a common nutritional disorder, is associated with diabetes, hypertension, hyperlipidemia, cancer and many other health related problems. Obese gene, leptin, mediates its effects via the Leptin receptor or obese receptors (**OBR** or **LR**) that is expressed in several tissues including hypothalamus. The OBR has at least 6 alternatively spliced forms (**OBRa-f** or **LRA-f**) that contain a common extracellular domain. OBRb or long isoform contains a unique ~ 300 aa long cytoplasmic tail that is required for leptin signaling. OBR is abnormally spliced (truncation of cytoplasmic domain) in db/db mice resulting into severe obesity.

Leptin is structurally related to cytokine family and it activates cytokine-like signal transduction by stimulating the JAK-STAT pathway via the OBRb, a member of IL-6 receptor family of class I cytokine receptors. Leptin binding to OBRb results in the activation of JAK2 and subsequent phosphorylation of specific tyrosines (**Tyr985** and **Tyr1138**) located at the C-terminal, cytoplasmic region of OBRb. Phosphorylation of OBRb **Tyr1138** recruits the SH2 domain containing STAT3, its autophosphorylation, translocation to the nucleus, and modulation of gene transcription, most notably induction of suppressor of cytokine signaling 3 gene (**SOCS3** or **CIS3**). SOCS3 inhibits leptin signaling. SOCS3 have been reported to be elevated in some obese animals. **Tyr985** phosphorylation of OBRb recruits the SH2 domain containing protein-tyrosine phosphatase **SHP-2**, it's binding to **GRB2**, and subsequent activation of **ERK**, a group of ser/thr kinases involved in the regulation of gene transcription. Therefore, OBRb Tyr phosphorylation plays in critical role in leptin signaling.

Sources of antigen and antibodies

An 11 aa sequence (**designated OBR14-P, phosphorylated peptide**) within the cytoplasmic C-terminus of mouse OBRb (980-990 aa; **C-QP SVK (Y)AT LVS; Tyr985 phosphorylated**) (1) was coupled to KLH and polyclonal antibodies generated in **rabbits**. **Control non-phosphorylated peptide (cat # OBR15-P)** was used to negatively adsorb the phosphopeptide antiserum and making it specific for the OBRb-Tyr985.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)

100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Affinity pure IgG

100 ug/100ul solution lyophilized powder
Supplied in **Buffer:** PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide

100 ug/100 ul solution lyophilized powder
Supplied in **Buffer:** PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Working antibody dilution (2-10 ug/ml) should be optimized and adjusted according to the sample composition, and technique employed (**Western blotting, immunoprecipitations, histochemistry**) and sensitivity of detection (colorimetric or Chemiluminescent substrates).

ELISA (0.1-2 ug/ml using 10-100 ng of coated peptide/well).

Specificity and crossreactivity

Mouse OBR14-P sequence is specific for the long isoform (OBRb). It is 100% conserved in rat, 91% in human, and monkey OBRb. The antibody is specific for OBRb phosphorylated at Tyr985. It does not react with non-phosphorylated OBRb or OBRb phosphorylated at Tyr 1138. Antibody cross-reactivity in various species has not been studied. Phosphorylated OBRb peptide (Cat # OBR14-P) and non-phosphorylated peptide (Cat # OBR15-P) can be used as suitable controls to confirm antibody specificity in various assay. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol see detailed protocol at the web site).

General References: Bjorbaek C et al (1999) J. Biol. Chem. 274, 30059; Bjorbaek C et al (2000) J. Biol. Chem. 275, 40649; Bank AS et al (2000) J. Biol. Chem. 275, 14563; Bjorbaek C et al (1998) Mol. Cell 1, 619; Tartaglia, LA (1995) Cell 83, 1263; Chen H et al (1996) Cell 84, 491-495; Lee GH et al (1996) Nature 379, 632-635; Cioffi JA et al (1996) Nat. Med. 2, 585-589

This product is for In vitro research use only.

Related material available from ADI

Recombinant Leptin, Antibodies to Leptin, Leptin receptor, leptin receptor (Y985) and Y1138, and OB-RGRP antibodies.
OBR14-S-A-P 71212A

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