Product Specification Sheet

Insulin regulated aminopeptidase (IRAP) Antibodies

Cat. # IRAP11-P	Rat IRAP Control/blocking Peptide #1	**SIZE**: 100 ug
Cat. # IRAP11-S	Rabbit Anti-rat IRAP antiserum #1	**SIZE**: 100 ul
Cat. # IRAP11-A	Rabbit Anti-rat IRAP IgG # 1 (aff pure)	**SIZE**: 100 ug

Tankyrases, **renamed as TNKS-1/TANK1,** a modular protein with homology to ankyrin and poly(adenosine diphosphate-ribose) polymerase (PARP) has been cloned and localized to telomere. TANK1 is targeted to pericentriolar domain of centrosomes during mitosis. After mitosis, TANK1 associates with the golgi as a peripheral membrane protein and implicated in targeting of GLUT4 vesicles. In the absence of insulin, GLUT4 vesicles reside in the Golgi and cytoplasm. Upon stimulation by insulin, GLUT4 vesicles undergo exocytosis and translocate to the plasma membrane. GLUT4 vesicles are characterized by the presence of glucose transporter 4 (GLUT4) and **IRAP (Insulin regulated aminopeptidase**). The exocytosis of GLUT4 vesicles is therefore plays an important role in uptake of glucose through Glut4 and the degradation of various vascoactive hormones by IRAP. TANKs have been colocalized in GLUT4 vesicles and shown to bind IRAP via the ANK-repeats. IRAP (also known as placental leucine aminopeptidase, PLAP; leucyl cystinyl aminopeptidase, LNPEP; oxytocinase, Otase; vesicle protein of 165 kDa or vp165) belongs to the peptidase M1 family. IRAP (mouse 966aa, rat 1025 aa; human 1025 aa, chromosome 5q14.2-q15) release an n-terminal amino acid, cleave before cysteine, leucine as well as other amino acids. IRAP, a zinc-containing metalloprotein, degrade peptide hormone such as oxytocin, vasopressin, and angiotensin III, inactivate Met-enkephalin and dynorphin. It is a type 2 membrane protein and also secreted due to proteolytic processing. **IRAP** is glycosylated and alternatively spliced to at least 3 isoform (1-15 aa missing in isoform 2 and 1-20 missing in isoform 3). The N-terminus (1-110 aa) is located in the cytoplasm, followed by TM domain (111-131 aa) and the C-terminus (132-1025 aa) is extracelluarl. The secreted form is cleaved at 154-155 aa (processed secreted form 155-1025 aa). IRAP is highly expressed in placenta, heart, kidney, and small intestine. In brain, only the membrane form is found. The brain form is found to be 140 kDa due to differential glycosylation.

Source of Antigen, Antibodies, and Positive Controls

Antigen	17-aa peptide from **rat IRAP/LCAP (protein accession # P97629, refs 1)**; **Designation (# IRAP11-P, control or blocking peptide**) conjugated to KLH; epitope ~ **extracellular** domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**IRAP11**-S) and IgG, purified over antigen-agarose (Cat # **IRAP11**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 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

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique). IRAP ~165 kDa; brain form 140 kda; secreted form ~150 kda.

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested. We recommend the use of affinity purified ab at 2-20 ug/ml.

Specificity & Cross-reactivity

The IRAP11 control peptide sequence is 82% conserved in mouse and 76% in human IRAP isoform 1 and 2. Antibody crossreactivity in various species is not established. 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 at: www.4adi.com\data\abblock.html).

General References: (1) Keeler SR et al (1995) JBC 270, 23612; masticl CC et al (1994) JBC 269, 6089; Chi N-W et al (2000) JBC 38437; Sbodio JI et al (2002) Biochem. J. 361, 451;

*This product is for In vitro research use only.

IRAP11-S-A-P 70901A

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