ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Ghrelin Receptor (GHS-R) Antibodies

Cat. GHSR11-S	Rabbit Anti-Rat GHS-R antiserum # 1	**SIZE**: **100 ul**
Cat. GHSR11-A	Rabbit Anti-Rat GHS-R IgG # 1(aff pure)	**SIZE**: **100 ug**
Cat. GHSR11-P	Rat GHS-R Control/blocking peptide # 1	**SIZE**: **100 ug**

GH secretagogues (GHSs) are synthetic compounds that are potent stimulators of GH release. GHSs, act through a novel orphan G-protein-coupled receptor (GPCR), the **GHS receptor (GHS-R)**. **Ghrelin** has been purified and identified from rat and human stomach as the endogenous ligand for the GHS-R. The rat and human mature Ghrelin (28-aa) are produced from 117 amino acids precursor. In rat stomach, a 28-aa **Ghrelin** and **des-Gln14-Ghrelin** (deletion of Gln14) are produced due to alternative splicing of Ghrelin mRNA. The activity of both Ghrelins is the same. Ghrelin has an unusual modification at Ser3 residue that is **n-octanoylated** and it is essential for biological activity. Rat Ghrelin is expressed in the stomach, small and large intestines, and brain regions (hypothalamic arcuate nucleus) that are involved the regulation of food intake. Both Ghrelin and GHS-R expression is detected in the heart, suggesting that Ghrelin might have some cardiovascular effects.

GHS-R (rat 364 aa; human 366 aa, ~90% sequence homology) is a receptor for GHRP and non-peptide, low mol wt. secretagogue. This receptor is coupled to G-a11. Two isoforms of GHS-R have been reported: isoform 1a (human 366 aa) and type 1b (289 aa and only 5 TM domains) appears not to bind the ghrelin. GHS-R displays the typical structure of GPCR with TM domains, extracellular N-terminus and intracellular C-terminus. It is expressed in not only in the pituitary and hypothalamus but also in the hippocampus, pancreas, and neuroendocrine tumors, including somatotropinomas and rat GH3 cells.

Source of Antigen and Antibodies

A 20 aa peptide sequence (designated **GHSR11-P or control peptide**) within the **cytoplasmic** C-terminus of **Rat GHS-R** (1) was synthesized, coupled to KLH and antibodies generated in **Rabbits**. Control peptide was also coupled to Sepharose for the **affinity purification** of antibodies.

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 using Chemiluminescence technique). An antibody made to the ERAB11 epitope has detected ~ 27 kDa protein in the brain.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence. We recommend the use of affinity purified antibody at 10-30 ug/ml in formaldehyde fixed, paraffin-embedded tissues (1).

Specificity & Cross-reactivity

The Rat GHSR11-P sequence is100% conserved in mouse, 95% conserved in human, sheep and pig GHS-R type 1a receptors. GHSR11-P sequence is not found in type 1b GHS-R. No significant sequence homology exists with other GPCRs. Antibody cross-reactivity in various species has not been studied. 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 the web site).

General References: (1) Howard AD et al (1996) Science 273, 974; kaji H et al (1998) JBC 273, 33885; Shibasaki SY et al (2001) Life Sci. 68, 991; McKee KK et al (1997) Mol. Endocrinol. 11, 415; Smith Rg et al (1999) Trends Endocrinol. Metabol. 10, 128; Guan XM et al (1997) Brain res. Mol. Brain. Res. 48, 128; Kojima M et al (1999) Nature 402, 656;

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This product is for in vitro research use only.

Some New Antibodies from ADI…

- Neuromedin U, NMUR1/2, NTR1-3 receptors, Motilin and Motilin receptor, Orexin and orexin receptors, CART, and Leptin receptors

Western Blot recycling kit (Use the same blot to probe with multiple antibodies) **recycle blot in 5-10 min.**

Study distribution of proteins in **pre-made protein blots of brain, kidney, GI-tract, and major tissues**

GHSR11-S-A-P 71212J

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