ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

EP3 Receptor Antibodies

Cat. # EP31-P	Rat EP3 Control (blocking) Peptide # 1	**SIZE**: 100 ug
Cat. # EP31-S	Rabbit Anti-Rat EP3 antiserum # 1	**SIZE**: 100 ul
Cat. # EP31-A	Rabbit Anti-Rat EP3 IgG # 1(affinity pure)	**SIZE**: 100 ug

Prostanoids are the cyclooxygenase metabolites derived from C-20 unsaturated fatty acids (arachidonic acid) and include prostaglandin (PG) D2, PGE2, PGF2 alpha, PGI2, and thrombaxane (Tx) A2. **Prostaglandin PGE2** is one of the major prostaglandin produced during inflammation. A variety of PGE2-mediated effects on vascular smooth muscle tonus, glomerular cell function, renin release, and renal salt and water transport have been described. The actions of PGE2 are mediated by rhodopsin-type; G-protein coupled membrane receptors, termed **E-prostanoid (EP) receptors or PTGERs**. There are four subtypes of PGE receptors designated as **EP1, EP2, EP3, and EP4** that are encoded by different genes and expressed differently in each tissue. The intracellular signaling also differs among the receptor subtypes. In general, EP receptors display a protein topology typical of GPCR - 7 TM domain, an extracellular N-terminus, and a large intracellular C-terminus.

EP3 (mouse 3-4 isoforms, 365-425 aa; rat 2 isoforms 364-365 aa; human 3-6 isoforms, 365-425 aa, chromosome 1p31.2,) is expressed in the kidney, brain, stomach, uterus, liver, skeletal muscle, intestine, and thymus. Alternative splicing of EP3 produces various isoforms (alpha, beta, gamma, etc) with different C-terminal tails. EP3 isoforms have identical ligand binding but different coupling properties with G-proteins: alpha and beta coupled to Gi, whereas gamma couples with Gi and Gs. EP3 knockout mice show impaired febrile response to pyrogens.

Source of Antigen and Antibodies

Antigen	15aa peptide of rat EP3; **Designated (EP31-P or control peptide /blocking peptide**) conjugated to KLH; epitope location ~ N-terminus, Extracellular domain
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#EP31-S) and IgG, purified over antigen-agarose (Cat # EP31-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 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). EP3 is ~62 kDa protein in the kidney (2).

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 antibody at 2-20 ug/ml as described (2) or paraformaldehyde fixed tissues.

Specificity & Cross-reactivity

The rat EP31-P control peptide is conserved in all EP3 isoforms that differ at the C-terminus. It is 75% conserved in mouse EP3-beta and –gamma. No significant homology of EP31-P exist with either human EP3 or other EP (1, 2 or 4) receptors. Antibody cross-reactivity in various species is not established. The EP31-P 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**. Sugimoto Y et al (1992) J. Biol. Chem. 267, 6463; Sugimoto Y et al (1993) J. Biol. Chem. 268, 2712; Irie A et al (1993) Eur. J. Biochem. 217, 313; Hasegawa H et al (1993) J. Biol. Chem. 271, 1857; Narumiya S et al (1999) Physiol. Rev. 79, 1193; Morath R et al (1999) J Am. Soc. Nephrol. 10, 1860.

2. Citations of for ADI Antibodies (see updates at the web site)

Walch L 2003, Endocrinology, 144: 1284 - 1291 WB
Aronff DM, 2004, J. Immunol., Jul 2004; 173: 559 – 565, WB
Biswas S 2004, Prostaglandins, Leukotrienes and Essential Fatty Acids, 71, 277-278, IHC
White ES, 2004, Am. J. Respir. Cell Mol. Biol, WB
Aronoff DM, 2004, J. Immunol.,173: 559 – 565, WB
Burgess JK, 2004, J Allergy Clin. Immunol. 113, 876-881,

*This product is for In vitro research use only.

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