ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Cycoloxygenase-2 (COX-2 PGHS-2;PHS-2;Prostaglandin-endoperoxide synthase-2)

Cat. COX23-M	Mouse monoclonal Anti-Human COX2 IgG # 3	**SIZE**: 100 ug
Cat. COX23-C	Human COX-2 Protein WB +ve control	**SIZE**: 100 ul

Prostaglandins are important regulators of immune responses, fever, and pain. Two isoforms of Prostaglandin H synthase are well characterized, namely COX1 (also called PGHS-1; PHS-1; Prostaglandin-endoperoxide synthase-1) and COX2 (also called PGHS-2; Prostaglandin-endoperoxide synthase-2 and PHSII). Both forms of COX proteins are membrane associated heme proteins containing Cyclooxygenase and peroxidase activities. These enzymes are targets of NSAID (Non steroidal anti-inflammatory drugs) such as aspirin. Cox-1 (human 599 aa; rat 602 aa) is homodimer of 70KD subunits (1). COX1 is constitutively expressed although significant enhancement of COX1 expression can be induced in some cell types. High expression is observed in gastrointestinal tissues. COX2 is a 72KD protein having 60% homology to COX1. It is induced by cytokines and mitogens and is likely to play a role in inflammatory diseases such as rheumatoid arthritis.

Source of Antigen and Antibodies

Antigen	Human Cox-2 was expressed and purified from sf21 cells
Ab Host/type	Balb/c **mouse**. Splenocytes were fused with Sp2/0 myeloma cells. Resulting clone (designated **COX23**, isotype IgG1), selected for reactivity with cox2, was expanded into mice as **ascites**. Antibody has been purified by Protein A/G column chromatography **(#COX23-M)**.
2-Ab	Goat Anti-mouse IgG-HRP conjugate Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve control IgG	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

Human Cox-2 was expressed and purified (80%, ~70 Kda) from sf21 cells. Partially purified, recombinant **human cox-2 protein** was formulated as **positive Control (cat #COX23-C)** for Western Blots. it is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **COX23**-C for good visibility with antibody Cat # **COX23**-M. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **COX23**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Form & Storage of Antibodies/Peptide Control

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/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 (1-10 ug/ml for affinity pure using Chemiluminescence technique). COX2 is ~70 kDa band in LPS+PMA induced RAW 264.7 cells and not in non-induced cells.

ELISA (1:10K-1:100K) using 100 ng antigen/well.

Histochemistry & Immunofluorescence: We recommend the testing of antibody at 2-20 ug/ml. Cells can be induced with LPS+PMA prior to fixing.

Specificity & Cross-reactivity

The COX23-M reacts with human and murine Cox-2. Other species not tested. There is no reactivity to purified COX-1. Purified Cox-2 (COX23-C) should be used a positive control for western.

General References: (1). Hla, T. & Neilson, K. (1992) *PNAS USA*, **89**, 7384-7388; Jones et al (1993) JBC 268, 9049; Yamagata K et al (1993) Neuron 11, 371;

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

DiPerna CA, 2003, J. Thoracic Cardiovascular Surgery, 126, 1129-1133, , IHC
DiPerna CA, 2002, Chest. 122(4) Supplement:165S-166S, , IHC
Carlson NG, 2006, Journal of Neuroimmunology, 174, 21-31, , IHC
Castle PE, 2003, Cancer Epidemiol. Biomarkers Prev., 12: 1449 - 1456, WB,

*This Product is for *in vitro* research use only.

Related materials available from ADI

COX-2 ELISA Kit

Cox23-M-C 71217A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com