ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3410	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. 100-200-CX2

Human Cyclooxygenase 2 (Cox-2)

ELISA KIT Cat. #100-200-CX2

For Quantitative Determination of Cox-2
In Human Cells/Tissues

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Human Cox-2 ELISA KIT Cat. # 100-200-CX2

Kit Components	96 tests
Anti-Human Cox-2 antibody coated strip plate (96 wells), **Cat. # 1 0 0 2 0 0 - 1**	1 plate
Anti-human Cox-2-HRP conjugate, 30X), 0.4 ml **Cat # 1 0 0 2 0 0 -2** (dilute 1:30 with conjugate diluent)	1 vial
ELISA Buffer (sample diluent) **Cat # 100200-3** (30 ml)	1 bottle
Purified recombinant Human **Cox-2 Standard Stock** (powder, Reconstitute. with 0.5 ml water to prepare 140 ng/ml stock) see detailed instruction; **Cat # 100200-4**	2 Vial
Chromogen (TMB); **Cat # 100200-5,** 15 ml	1 bottle
Stop Solution (1N H2SO4) **cat # 100200-6 (12 ml)**	1 bottle
Wash Buffer (40X) Dilute 1:40 before use **Cat # 100200-7 (50 mls)**	1 bottle
Conjugate Diluent, 12 ml, **Cat # 100200-8**	1 bottle
Instruction Manual, 1 0 0 - 2 0 0 - C X 2	1

Introduction

Prostanoids sensitize peripheral nociceptor terminals and produce localized pain hypersensitivity. The prostanoid family includes PGD2, PGE2, PGF2alpha, PGI2, thromboxane A2 and prostaglandins. The prostaglandins (PGs) are implicated in various physiological and pathophysiological events, including male fertility, menstruation, ovulation, pregnancy, implantation and inflammatory and neoplastic diseases. The biosynthesis of PGs and some other prostanoids, is catalyzed in a rate limiting step by PG-H synthase (also known as cyclooxygenase (COX), PG-endoperoxidase synthase (PTGS)) which converts arachiodonic acid to prostaglandin/prostanoid precursor PGH2. Two cyclooxygenase isozymes, COX1 (human, 576aa, 69-72kDa; chromosome 9) and COX2 (human, 604aa, 74 kDa; chromosome 1) have been identified. COX1, a constitutively expressed isoform, produces physiologically relevant prostanoids such as those in stomach and platelets. COX2 is a 74 kDa protein having 60% homology with COX1, expressed by extracellular stimuli such as tumor promoters, pro-inflammatory cytokines, mitogensand oncogene in different cells. The control of COX2 gene expression is regulated at the level of transcriptional and post-transcriptional mechanisms. COX2 isoform is inducible and rapidly upregulated at inflammation sites and forms proinflamatory prostanoids. The overexpression of COX-2 also leads to tumorigenesis. Recently, a third isoform COX3 (canine 633aa; ~65kDa in human aorta) has been reported. Two smaller COX1-derived proteins (partial COX1) PCOX1a (canine 414aa, ~53kDa in human aorta) and PCOX1b have also been characterized. The COX3, but not PCOX1a, possesses glycosylation-dependent cyclooxygenase activity. The nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the formation of prostaglandins by inhibiting the activity of cyclooxygenases (COX1, COX2 and COX3), this ability was associated with inhibition of COX, which converts arachidonic acid to the prostaglandin precursor prostaglandin H2.

ADI's COX-2 ELISA is designed to measure human Cox-2 in culture media, cells or tissue extracts. It is not recommended for serum or plasma due to low concn of Cox-2.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standard, the minimum cox-2 concentration detectable using this assay 0.25 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Three samples were run in 10 duplicates. The samples showed good intra-assay precision (2-7 % CV). The actual values were: mean 139.9 ng/ml (SD 3.46 ng/ml); 52.8 ng (SD 2.97) and 6.4 ng/ml, SD 0.49 ng/ml).

Inter-assay precision: Three samples (137, 52, 6.5 ng/ml) were run in duplicate in 10 independent assays. The samples showed good inter-assay precision (4-6 % CV). The actual values were: mean 137.9 ng/ml, SD 8.28 ng/ml; 52.5 ng (SD 3.12) and 6.2 ng/ml, SD 0.30 ng/ml).

3. LINEARITY

A sample (with original Cox-2 concentration of 137.5 was diluted (1:2, 1:4, 1:8, 1:16, 1:32, and 1:64) and the cox-2 values determined. The samples showed excellent mean recoveries of about 98%, respectively (range 89-114%).

5. SPECIFICITY

The antibodies used in this kit are specific for human Cox-2. No significant reactivity is seen with Cox-1. Suitability of the human Cox-2 kit with other species (Mouse or rat Cox-2) has not been determined.

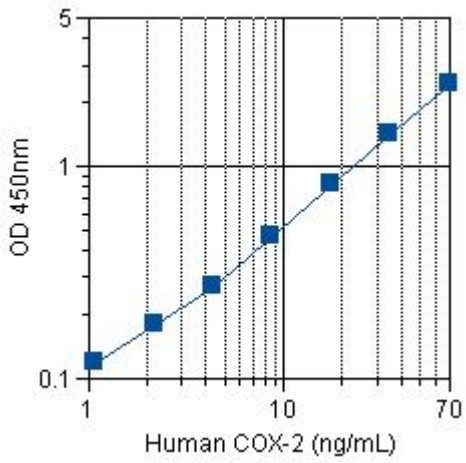
6. TITER ASSAY

A standard was serially diluted 1:2 with the ADI Cox-2 lysis buffer (#100-200-9; available separately) and measured in the ELISA. The sample (34.23, 18.58, 9.58, 4.48 ng/ml) showed excellent mean recoveries of about 98%, respectively (range 98-109%).

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Av. A_{450nm}	Calculated concn.
A1, A2	**Std. A** (70.00 ng/ml)	2.54	
B1, B2	**Std. B** (35.00 ng/ml)	1.52	
C1, C2	**Std. C** (17.5 ng/ml)	0.82	
D1, D2	**Std. D** (8.75 ng/ml)	0.47	
E1, E2	**Std. E** (4.38 ng/ml)	0.28	
F1, F2	**Std. F** (2.19 ng/ml)	0.17	
G1, G2	**Std. G** (1.09 ng/ml)	0.14	
H1, H2	**Std blank** (0 ng/ml)	0.09	
A3, A4	**Sample 1**	0.81	17.2 ng/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Human Cox-2 ELISA kit is based on binding of human Cox-2 from standards or samples to mouse monoclonal antibody coated on the plate and anti cox-2-HRP conjugate. Higher concentrations of Cox-2 in the sample result in increased binding of anti cox-2-enzyme (HRP) to the antibody coated plate. After a washing step, chromogenic substrate (TMB) is added and colors (blue) developed. The enzymatic reaction (color) is directly proportional to the amount of Cox-2 present in the sample. Adding stopping solution terminates the reaction (blue color turns yellow). Absorbance is then measured using an ELISA reader at 450 nm. and the concentration of Cox-2 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

ADI's Cox-2 ELISA kit is intended for *in vitro research* use only. The reagents contain cetylpyidinium chloride as preservative of conjugate and sodium azide in the standards. Appropriate care should be taken when disposing solutions. The stds./controls sera may contain human serum that has been shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Sample Preparation

1. Total cells or tissue lysate or culture media are recommended samples for the measurement of cox-2. We do not recommend assaying COX-2 in serum or plasma due to very low concentration of Cox-2 in these samples.
2. **Preparation of lysate**: Determine total cell count and wash cells or tissue with cold PBS pH 7.4 or normal saline to remove traces of blood/serum or culture media. Collect cells by scraping or centrifugation. Followin protocol is recommended for approx. 10 million cells ($1x10^7$ cells): Add 500ul of TNE buffer (10mM Tris, pH 8.0, 1% NP-40, 0.15M NaCl, 1mM. EDTA) and then sonicate (Repeat 5 times, 30 sec. sonication and 1min remains). Centrifuge the cell lysate for 5 min at 15000 rpm, and collect supernatant. Clear supernatant (undiluted or 5-10 diluted) can be used for measuring Cox-2. Tissues can be homogenized in the lysate buffer as buffer, followed by centrifugation. RIPA buffer can also be used for preparing cell lysate. Buffers containing CHAPS or Sucrose Monolaurate are not recommended.
3. Determine total protein concentration is Cox-2 concn is expressed as per mg of total protein.
4. Cell lysate should be kept at 4oC for immediate assay or frozen at -80oC. It is preferable to determine Cox-2 in freshly prepared lysate.
5. ADI's Cox-2 lysis buffer can be purchased separately #200-100-9.
6. Sample should be measured immediately after preparation or frozen at -20oC or below. Do not freeze and thaw samples.
7. Samples should be prepared in lysis buffer or diluted at least 1:4 with the ELISA buffer.
8. Use test samples in neutral pH range. Use of solvents may affect the assay.

REAGENTS PREPARATION FOR THE ASSAY

1. Preparation Human Cox-2 Standards: Reconstitute stock Cox-2 standards in 0.5 ml water (concn 140 ng/ml). Perform a 1:2 serial dilution with ELISA buffer # 100200-3 as follows:

	ELISA Buffer #3	Dilution	Final Concn	Std Name
225 ul stock	225 ul	1:2	70 ng/ml	A
225 ul of Std A	225 ul	1:4	35 ng/ml	B
225 ul of Std B	225 ul	1:8	17.5 ng/ml	C
225 ul of Std C	225 ul	1:16	8.75 ng/ml	D
225 ul of Std D	225 ul	1:32	4.38 ng/ml	E
225 ul of Std E	225 ul	1:64	2.19 ng/ml	F
225 ul of Std F	225 ul	1:128	1.09 ng/ml	G
Blank	225 ul	none	0 ng/ml	0 (blank)

When preparing serial dilution, make sure that the buffer and standards are mixed be gentle vortexing before taking aliquots for the next dilution. A total of 200 ul will be used for each run (100 ul used in duplicate). Prepare standards fresh and do not store for more than a few hours at 4oC. Reconstituted stock can be frozen at -20oC to -80oC for later use.

2. **Wash buffer Dilution**: Dilute stock (40X) wash buffer 1:40 with water (25 ml stock and 975 mls of water; mix it thoroughly. It can be kept at 4oC for 2-4 weeks. Wash buffer 1X= PBS pH 7.5 and 0.05% Tween-20.

3. **Cox-2 antibody-HRP Conjugate** # 100200-2 is supplied as 30x stock. Dilute 1:30 with conjugate diluent # 100200-8 in required amounts only (need ~800 ul per strip of 8-wells; take 30 ul stock and 870 of diluent; For a full plate (300 ul of stock and 8.7 ml of diluent) and mix it gently at room temp for 5 min. The conjugate must be prepared before the assay. It can be kept at room temp for 1-2 hrs or 4oC for 1-4 days at 4oC.

Assay Procedure

Allow **all** reagents to reach room temperature; arrange and label required # of strips. Dilute wash buffers, antibody conjugate, standard stock, and prepare Stds.

1. Use ELISA buffer (100 ul) as blank. Pipet **100 ul of standards** and **samples** in duplicate into appropriate wells. Mix gently. Cover the plate and incubate at **37oC temp. for 60 min**.

2. **Wash 4-times** with diluted wash buffer (300 ul/well/wash) if washing manually. Remove traces of liquid by tapping it over paper towels between each wash.

3. Pipet **100 ul diluted anti-Cox2-HRP conjugate**. Mix gently. Cover the plate and **incubate at 4oC for 30 minutes**

4. **Wash 7-times** as in Step 2. Remove traces of liquid by tapping the plate over clean paper towels.

5. **Add 100 ul TMB substrate**, mix gently **and incubate at room temp for 30 min**. Blue color develops in standards and positive wells..

6. Add **100 ul stop solution** into each well and mix gently. (Blue color turns yellow). Measure absorbance at 450nm within 30 minutes. Calculate unknown values using standard curve.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Due to the susceptibility of the some assay components, it is recommended that the entire kit is used immediately after reconstitution of the components or the components used within a few days.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing >140 ng/ml Cox-2 should be diluted with the ELISA buffer (sample diluent) #100200-3. The results obtained should be multiplied by the appropriate dilution factor. If samples are too dilute, i.e. It may be necessary to test the samples at several dilution to determine optimum dilution. below the detection level, it may be necessary to prepare more concentrated cell lysate.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the blank from all stds and sample wells. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate Cox-2 concentrations. Use 4-point curves (point-to-point and do not force a regression or straight line) Read off Cox-2 concentrations of the control and patient samples.