COX-2 (human), ELISA kit

Catalog No. ADI-900-094

96 Determination Kit

Table of Contents

Description	Page 2
Introduction	2
Precautions	2
Materials Supplied	3
Storage	3
Materials Needed but Not Supplied	3
Sample Handling	4
Procedural Notes	4
Reagent Preparation	5
Assay Procedure	6
Calculation of Results	7
Typical Results	7
Typical Standard Curve	8
Performance Characteristics	9
Sample Dilution Recommendations	10
References	11
Limited Warranty	12

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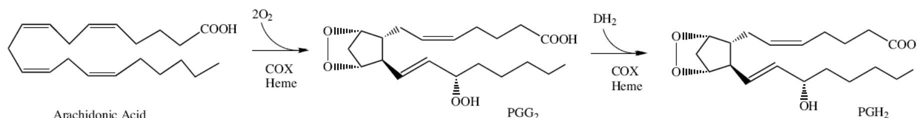
Description

The COX-2 (human), ELISA kit is a complete kit for the quantitative determination of human COX-II in cell culture lysates. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to human COX-II immobilized on a microtiter plate to bind the human COX-II in the standard or sample. A recombinant human COX-II Standard is provided in the kit. After a short incubation the excess standard or sample is washed out and a rabbit polyclonal antibody to human COX-II labeled with the enzyme Horseradish peroxidase is added. This labeled antibody binds to the human COX-II captured on the plate. After a short incubation the excess labeled antibody is washed out and substrate is added. The substrate reacts with the labeled antibody bound to the human COX-II captured on the plate. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of human COX-II in either standards or samples. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Cyclooxygenase (COX, also known as Prostaglandin G/H synthase) is a membrane bound enzyme responsible for the oxidation of arachidonic acid to Prostaglandin G_2 (PGG_2) and the subsequent reduction of PGG_2 to PGH_2[3,4]. The conversion is shown below. These reactions are the first steps in the formation of a variety of prostanoids. COX has been shown to be expressed in at least two different isoforms, a consitutively expressed form, COX-I, and an inducible form, COX-II. COX-I is thought to regulate a number of 'housekeeping' functions, such as vascular hemostasis, renal blood flow, and maintenance of glomerular function[5]. Inflammation mediators such as growth factors, cytokines and endotoxin induce COX-II expression in a number of cellular sytems[6,7]. The effect of various non-steroidal anti-inflammatory drugs (NSAID's) on the activity of COX-I and -II is an area of considerable interest[8].

The Cyclooxygenase Reaction



Precautions

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1. Stop Solution is a 1 normal (1N) sulfuric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles, such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of samples, however, it is possible that high levels of interfering substances may cause variation in assay results.
4. The human COX-II Standard provided, Catalog No. 80-0908, should be handled with care because of the known and unknown effects of cyclooxygenase.

Materials Supplied

1. **human COX-II Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0906**
   A strip microtiter plate coated with mouse antibody specific to human COX-II.
2. **human COX-II Labeled Antibody, 1 vial, Catalog No. 80-0907**
   Rabbit antibody to human COX-II conjugated to Horseradish peroxidase.
3. **Assay Buffer, 30 mL, Catalog No. 80-0170**
   Phosphate buffered saline containing proteins and detergents.
4. **Labeled Antibody Diluent, 10.5 mL, Catalog No. 80-0182**
   Phosphate buffered saline containing proteins and detergents.
5. **Wash Buffer Concentrate, 50 mL, Catalog No. 80-0171**
   Phosphate buffered saline containing detergents.
6. **human COX-II Standard, 2 vials, Catalog No. 80-0908**
   A vial containing 275 ng of recombinant human COX-II.
7. **TMB Substrate, 15 mL, Catalog No. 80-1342**
   A solution of 3,3',5,5' tetramethyl benzidine (TMB) and hydrogen peroxide. Ready to use.
8. **Stop Solution, 11 mL, Catalog No. 80-0176**
   A 1N solution of sulfuric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **human COX-II Assay Layout Sheet, 1 each, Catalog No. 30-0160**
10. **Plate Sealer, 2 each, Catalog No. 30-0012**

Storage

All components of this kit, are stable at 4°C until the kit's expiration date.

Materials Needed but Not Supplied

1. Deionized or distilled water. No difference in assay results are seen with distilled water.
2. Precision pipets for volumes between 100 µL and 1,000 µL.
3. Repeater pipet for dispensing 100 µL.
4. Disposable beaker for diluting buffer concentrates.
5. Graduated cylinders.
6. A 37°C incubator.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 450 nm, preferably with correction between 570 and 590 nm.
9. Graph paper for plotting the standard curve.

Sample Handling

The COX-2 (human), ELISA kit is compatible with COX-II samples in cell lysates of human origin. This assay is not for use in serum or plasma due to the low levels of COX-II in circulation. Samples diluted sufficiently into Assay Buffer (≥1:2) can be read directly from the standard curve. Please refer to the Sample Recovery recommendations on page 10 for details of suggested dilutions.

Samples can be prepared by sonicating cells in TNE (10 mM Tris, pH 8.0, 0.15 M NaCl, 1% NP-40, 1mM EDTA) or RIPA (25 mM Tris, pH 7.4, 0.15 M KCl, 1% NP-40, 5 mM EDTA, 0.5% Sodium deoxycholate, 0.1% SDS) buffer. Five cycles of 30 second bursts at 1 minute intervals on ice is usually adequate for disruption. For optimal results, a small aliquot of the lysate should be examined under a microscope to verify sample disruption. The supernatant is collected after a 5 minute centrifugation at 15,000 rpm, 4˚C and assayed immediately. Typically, the supernatant from a lysate of 10^7 cells in 0.5 mL buffer will need an additional 5 to 10 fold dilution prior to the assay. Buffers containing CHAPS and sucrose monolaurate should be avoided.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses plates with removable strips. Unused strips must be kept desiccated at 4˚C in the sealed foil bag. The strips should be used in the frame provided.
8. **Prior to addition of standard, antibody, and substrate, ensure that there is no residual wash buffer in these wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. **Wash Buffer**

   Prepare Wash Buffer by diluting 25 mLs of the supplied concentrate with 975 mLs of deionized water. This can be stored at 4˚C until the kit expiration, or for 3 months, whichever is earlier.

2. **human COX-II Standard**

   Add 500 µL of deionized water to the human COX-II Standard. Let it sit at room temperature for 5 minutes. Mix it gently. This solution contains 140 ng/mL human COX-II.

   Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 230 µL of Assay Buffer into tube #1. Pipet 230 µL of Assay Buffer into tubes #2 through #7. Add 230 µL of the 140 ng/mL standard to tube #1. Vortex. Add 230 µL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #7.

   The concentration of human COX-II in tubes #1 through #7 will be 70, 35, 17.5, 8.75, 4.38, 2.19, 1.09 ng/mL respectively. See human COX-II Assay Layout Sheet for dilution details. STORE STANDARD AT -20˚C, avoid repeated freeze/thaws.

3. **Preparation of Labeled Antibody Conjugate**

   Add the entire contents of one (1) bottle of Labeled Antibody Diluent to the vial of human COX-II Antibody Conjugate. Let it stand at room temperature for 5 minutes and then vortex it gently. After reconstitution any unused Labeled Antibody should be aliquoted and stored at -20°C.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining strips with the desiccant back into the foil pouch and seal the ziploc. Store unused strips at 4°C.
2. Pipet 100 µL of Assay Buffer into the Blank wells.
3. Pipet 100 µL of Assay Buffer into the S0 (0 ng/mL Standard) wells.
4. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
5. Pipet 100 µL of the Samples into the appropriate wells.
6. Tap the plate gently to mix the contents.
7. Seal the plate and incubate at 37˚C for 1 hour.
8. Empty the contents of the wells and wash by adding 200 µL of wash solution to every well. Repeat the wash 6 more times for a total of **7 washes**. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint-free paper towel to remove any remaining wash buffer.
9. Pipet 100 µL of the Labeled Antibody into each well, except the Blank.
10. Seal the plate and incubate at 4˚C for 30 minutes.
11. Empty the contents of the wells and wash by adding 200 µL of wash solution to every well. Repeat the wash 8 more times for a total of **9 washes**. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint-free paper towel to remove any remaining wash buffer.
12. Add 100 µL of the TMB Substrate to each well.
13. Incubate for 30 minutes at room temperature in the dark.
14. Add 100 µL of Stop Solution to each well.
15. Blank the plate reader against the Blank wells, read the optical density at 450nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the blank wells from all readings.

Calculation of Results

Several options are available for the calculation of the concentration of human COX-II in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of human COX-II can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Plot the Average Net OD for each standard versus human COX-II concentration in each standard. Approximate a straight line through the points. The concentration of human COX-II in the unknowns can be determined by interpolation.

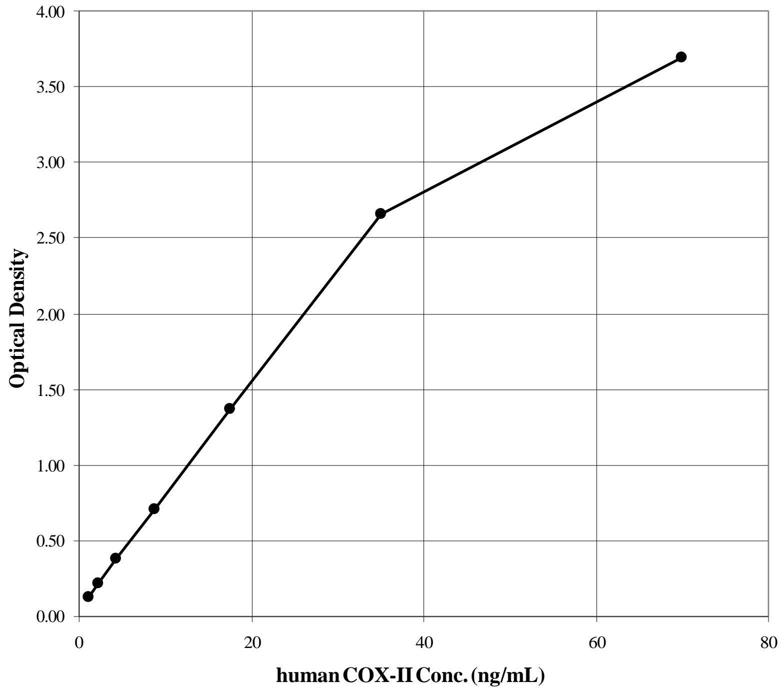
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	**human COX-II (ng/mL)**
Blank	0.060		
S1	3.689	3.629	**70**
S2	2.658	2.598	**35**
S3	1.367	1.307	**17.5**
S4	0.705	0.645	**8.75**
S5	0.383	0.323	**4.38**
S6	0.220	0.160	**2.19**
S7	0.129	0.069	**1.09**
Unknown 1	2.645	2.585	**24.53**
Unknown 2	0.262	0.202	**4.0**

Typical Standard Curve

The typical standard curve shown below **must not** be used to calculate human COX-II concentrations; the user must run a standard curve for each plate used.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[9].

Sensitivity

The sensitivity of the assay, defined as the concentration of hum an COX-II measured at 2 standard deviations from the mean of multiple zeros along the standard curve, was determined to be 0.25 ng/mL.

Linearity

A sample containing 104.45 ng/mL human COX-II was diluted 5 times 1:2 into the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual human COX-II concentration versus measured human COX-II concentration.

The line obtained had a slope of 0.8955 and a correlation coefficient of 1.000.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of human COX-II and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of human COX-II in multiple assays (n=3).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of human COX-II determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	human COX-II (ng/mL	Intra-assay %CV	Inter-assay %CV
Low	3.9	6.4	
Medium	10.7	8.4	
High	31.4	9.6	
Low	3.5		12.6
Medium	10.0		5.5
High	31.4		6.2

Cross Reactivities

The human COX-II ELISA kit is specific for human COX-II. The cross reactivity to human COX-I is <0.1%.

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Human COX-II concentrations were measured in TNE buffer. TNE buffer was diluted 1:2 in the assay buffer and spiked to concentrations throughout the dynamic range of the assay. The following results were obtained:

Sample	% Recovery *	Recommended Dilution *
TNE Buffer	94.4	1:2

* See Sample Handling instructions on page 4 for details.

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