COX-2 ELISA

Enzyme immunoassay for the quantitative determination of human COX-2 (cyclooxygenase-2) in cell lysate

REF **JP27186**

Σ **12 x 8**

For illustrative purposes only.
To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

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Code No. 27186

Human COX-2 Assay Kit

INTRODUCTION

Cyclooxygenase (COX) is a membrane bound enzyme responsible for the oxidation
of arachidonic acid to Prostaglandin G2 (PGG2) and the subsequent reduction of
PGG2 to PHG2. 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 constitutively expressed form, COX-1, and an inducible form, COX-2. COX-1 is
thought to regulate a number of housekeeping functions, such as vascular hemostasis, renal blood flow, and maintenance of glomerular function. Inflammation
mediators such as growth factors, cytokines and endotoxin induce COX-2 expression in a number of cellular systems.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies.
Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Hhuman COX-2.

MEASUREMENT RANGE

1.09 ～ 70 ng/mL

INTENDED USE

■ The Human COX-2 Kit is capable of the determination of Human COX-2 in lysate of cultured cells.
(Protocol example)
For cultured cells
1) Put cultured cells ($1x10^5$-$1x10^7$ cells) in 0.5 mL of IBLysis-I (product code #19022).
2) Mix thoroughly on a Vortex or by pipetting.
3) Solubilize by rotation at 2-8°C for 30 minutes.
4) Centrifuge at 10,000 rpm for 10 minutes at 2-8°C.
5) Apply the supernatant diluted by “4, EIA buffer” as necessary.

■ In the assay for cell lysate, it is recommend that the same number of cells is used.
■ It is recommend that total protein concentration is assayed at the same time in order to determine the proportion of Human COX-2 to total protein.

KIT COMPONENT

1 Precoated plate : Anti-Human COX-2 (13H14) Mouse IgG MoAb Affinity Purify 96Well x 1
2 Labeled antibody Conc.
: (30X) HRP conjugated Anti- Human COX-2 Rabbit IgG Fab’ Affinity Purify 0.4mL x 1
3 Standard : Recombinant Human COX-2 0.5mL x 2
4 EIA buffer 30mL x 1
5 Solution for Labeled antibody : 1% BSA, 0.05% Tween20 in PBS 12mL x 1
6 Chromogen : TMB solution 15mL x 1
7 Stop solution : 1N H_2SO_4 12mL x 1
8 Wash buffer Conc. : (40X) 0.05% Tween20 in phosphate buffer 50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

・Plate reader (450nm) ・Micropipette and tip
・Graduated cylinder and beaker ・Deionized water
・Refrigerator (as 4°C) ・Graph paper (log/log)
・Paper towel ・Tube for dilution of Standard
・Incubator (37°C ± 1°C)
・Washing bottle for precoated plate
・Disposable test tube for “2, Labeled antibody Conc.” and “6, Chromogen”

2. Preparation

1) Preparation of wash buffer
“8, Wash buffer Conc.” is a concentrated (40X) buffer. Adjust the temperature of “8, Washing buffer Conc.” to room temperature and then, mix it gently and completely before use. Dilute 50 mL of “8, Wash buffer Conc.” with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
“2, Labeled antibody Conc.” is a concentrated (30X). Dilute “2, Labeled antibody Conc.” with “5, Solution for Labeled antibody” in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.

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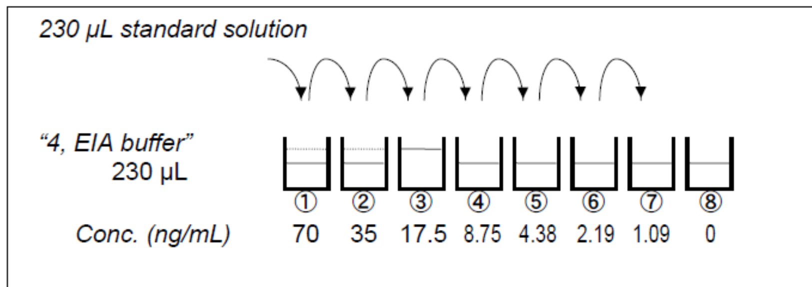
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of “2, Labeled antibody Conc.” with 870 μL of “5, Solution for Labeled antibody” and mix it. And use the resulting solution by 100 μL in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining “2, Labeled antibody Conc.” should be stored at 4℃ in firmly sealed vial.

3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of “3, Standard” and mix it gently and completely. This solution is 140 ng/mL Human COX-2 standard.

4) Dilution of Standard
Prepare 8 tubes for dilution of “3, Standard”. Put 230 μL each of “4, EIA buffer” into the tube.
Specify the following concentration of each tube.”
Tube-1 70 ng/mL
Tube-2 35 ng/mL
Tube-3 17.5 ng/mL
Tube-4 8.75 ng/mL
Tube-5 4.38 ng/mL
Tube-6 2.19 ng/mL
Tube-7 1.09 ng/mL
Tube-8 0 ng/mL (Test Sample Blank)
Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 70 ng/mL and 1.09 ng/mL.
Tube-8 is the test sample blank as 0 ng/mL.
See following picture.



5) Dilution of test sample
Test sample may be diluted with “4, EIA buffer” as necessary.

3. Measurement procedure
All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation for 60 minutes at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 9 times				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 μL each of “4, EIA buffer” into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash each well of the precoated plate vigorously with wash buffer using the mwashing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated

more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner as 4).
8) Take the required quantity of “6, Chromogen” into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to “6, Chromogen” bottle to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark.
The liquid will turn blue by addition of “6, Chromogen”.
10) Pipette 100 µL of “7, Stop solution” into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of “7, Stop solution”.
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of “7, Stop solution”.

SPECIAL ATTENTION

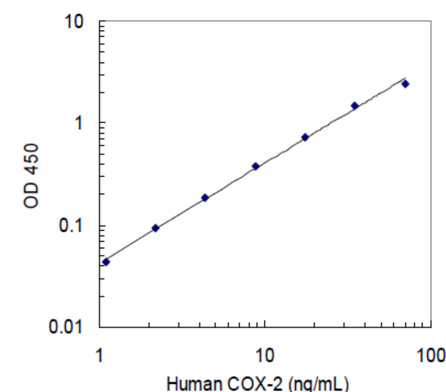
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need arises.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) “6, Chromogen” should be stored in the dark due to its sensitivity against light.
“6, Chromogen” should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and
unknown samples before plotting. Plot the subtracted absorbance of the standards
against the standard concentration on log-log graph paper. Draw the best smooth
curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (ng/mL)	Absorbance (450nm)
70	2.545
35	1.580
17.5	0.823
8.75	0.479
4.38	0.283
2.19	0.191
1.09	0.142
0 (Test Sample Blank)	0.098



＊ The typical standard curve is shown above. This curve can not be used to
derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
IBLysis-I	2	34.23	35.00	97.8
	4	18.58	17.50	106.1
	8	9.56	8.75	109.2
	16	4.48	4.38	102.2

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
IBLysis-I (x2)	17.50	16.23	92.7
	8.75	7.77	88.8
	4.38	4.19	95.6
	2.19	2.20	100.4

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
31.41	3.02	9.6	24
10.65	0.90	8.4	24
3.90	0.25	6.4	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
31.40	1.97	6.2	3
10.01	0.55	5.5	3
3.49	0.44	12.6	3

5. Sensitivity
0.25 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. “1, Precoated plate” and “3, Standard” contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
5. Precipitation may occur in “2, Labeled antibody Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The term of validity : 12 months
(The expiry date is specified on outer box.)

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