NEUROMICS

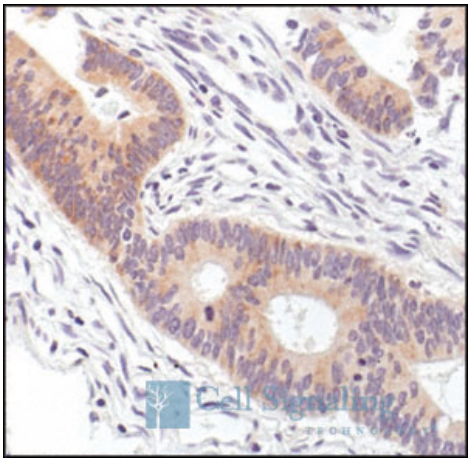
COX-2 Data Sheet

Catalog Number:	RA18005	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human and Mouse
Immunogen Sequence:	KLH coupled synthetic peptide derived from the sequence of human Cox2.	**Format:**	Liquid in 10mM sodium HEPES (pH7.5), 150mM NaCl, 100ug/ml BSA and 50% glycerol.
Applications:	Immunohistochemistry: 1:50 (Paraffin) Western Blot: 1:1000 (74 kDa) Flow Cytometry:1:100 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Maintain at +2-8 °C for 3 months or at -20 °C for longer periods. Stable for 1 year. Serum may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20ºC. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		

Application Notes

Description/Data:
Cyclooxygenase (COX), also known as prostaglandin G/H synthase, is a membrane bound enzyme partly responsible for the catalysis of prostanoid synthesis. COX is expressed as at least three different isoforms. COX-1 is constitutively expressed and thought to regulate a number of 'housekeeping' functions such as vascular hemostasis, renal blood flow, and glomerular function. COX-2 expression is tightly regulated and induced by inflammatory mediators such as growth factors, cytokines, and endotoxin. COX-3 appears to be much more strictly regulated spatially and is observed in greatest abundance in cerebral cortex.

Image: COX-2 staining of paraffin-embedded human colon carcinoma.



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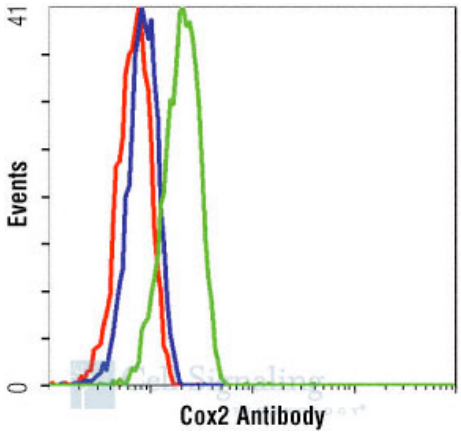

Image: Flow cytometric analysis of Raw cells, untreated (blue) or LPS-treated (green), using Cox2 antibody compared to a nonspecific negative control antibody (red).

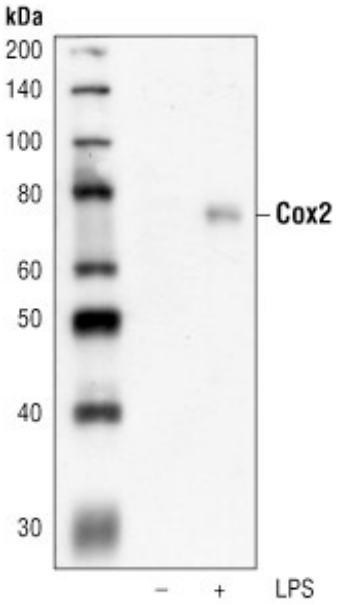

Image: Western blot analysis of extracts from Raw264.7 cells, untreated or LPS-treated (1 µg/ml for 6 h), using Cox2 Antibody.

Specificity:
Cox2 Antibody detects endogenous levels of total Cox2 protein.

Immunohistochemistry Protocol (Paraffin)

***IMPORTANT:** See below for the appropriate antibody diluent and antigen unmasking procedure.
IHC Protocol: Unmasking buffer/antibody diluent.
A Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH2O)
4. Hematoxylin (optional)
5. Wash Buffer:
1X TBS/0.1% Tween-20 (1X TBST): To prepare 1 L add 100 ml 10X TBS to 900 ml dH2O. Add 1 ml Tween-20 and mix.
10X Tris Buffered Saline (TBS): To prepare 1 L add 24.2 g Trizma® base (C4H11NO3) and 80 g sodium chloride (NaCl) to 1 L dH2O. Adjust pH to 7.6 withmconcentrated HCl.
6. *Antibody Diluent:
a. SignalStain® Antibody Diluent #8112
b. TBST/5% normal goat serum: To 5 ml 1X TBST add 250 µl normal goat serum.
c. PBST/5% normal goat serum: To 5 ml 1X PBST add 250 µl normal goat serum.

9. Blocking Solution: TBST/5% normal goat serum: to
1X PBS/0.1% Tween-20 (1X PBST): To prepare 1L add 100 mL 10X PBS to 900 mL dH20. Add 1 ml Tween-20 and mix.
10X Phosphate Buffered Saline (PBS): To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phophate, dibasic (Na2HPO4) and 2.4 g potassium phosphate, monobasic (KH2PO4) to 1 L dH2O. Adjust pH to 7.4.
7. *Antigen Unmasking:
a. Citrate: 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate (C6H5Na3O7•2H2O) to 1 L dH2O. Adjust pH to 6.0.
b. EDTA: 1 mM EDTA: To prepare 1 L add 0.372 g EDTA (C10H14N2O8Na2•2H2O) to 1 L dH2O. Adjust pH to 8.0.
c. TE: 10 mM Tris/1 mM EDTA/0.059 Tween–20, pH 9.0: To prepare 1L add 1.21 g Trizma® base (C4H11NO3) and 0.372 g EDTA (C10H14N2O8Na2•2H20) to 950 ml dH2O. Adjust pH to 9.0, add 0.5 ml Tween–20, then adjust final volume to 1000 ml with dH2O.
d. Pepsin: 1 mg/ml in Tris-HCl pH 2.0.
8. 3% Hydrogen Peroxide: To prepare, add 10 ml 30% H2O2 to 90 ml dH2O.

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5ml 1X TBST add 250 µl normal goat serum.
10. Biotinylated secondary antibody.
11. ABC Reagent: (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
12. DAB Reagent or suitable substrate: Prepare according to manufacturer's recommendations.

B Deparaffinization/Rehydration
NOTE: Do not allow slides to dry at any time during this procedure.
1. Deparaffinize/hydrate sections:
a. Incubate sections in three washes of xylene for 5 minutes each.
b. Incubate sections in two washes of 100% ethanol for 10 minutes each.
c. Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH2O for 5 minutes each.

*C *Antigen Unmasking*
NOTE: Consult product data sheet for specific recommendation for the unmasking solution.
1. For Citrate: Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then
maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top
for 30 minutes.
2. For EDTA: Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes
at a sub-boiling temperature. No cooling is necessary.
3. For TE: Bring slides to a boil in 10 mM TE/1mM EDTA/0.059 Tween-20, pH 9.0
then maintain at a sub-boiling temperature for 18 minutes. Cool on the bench
for 30 minutes.
4. For Pepsin: Digest for 10 minutes at 37°C.

D Staining
1. Wash sections in dH2O three times for 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections in dH2O twice for 5 minutes each.
NOTE: Consult product data sheet for recommended antibody diluent.
4. Wash section in wash buffer for 5 minutes.
5. Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100-400 µl primary antibody diluted in recommended antibody diluent to each section. Incubate overnight at 4°C.
7. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
8. Add 100-400 µl biotinylated secondary antibody, diluted in TBST per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
9. If using ABC avidin/biotin method, prepare ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
10. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
11. Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
12. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
13. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
14. As soon as the sections develop, immerse slides in dH2O.
15. If desired, counterstain sections in hematoxylin per manufacturer's instructions.
16. Wash sections in dH2O two times for 5 minutes each.
17. Dehydrate sections:
a. Incubate sections in 95% ethanol two times for 10 seconds each.
b. Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
c. Repeat in xylene, incubating sections two times for 10 seconds each.
18. Mount coverslips.

Western Blotting Protocol

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Solutions and Reagents for Western Blot
Note: Prepare solutions with Milli-Q or equivalently purified water.
1X Phosphate Buffered Saline (PBS)
1X SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)

Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
10X Tris Buffered Saline (TBS):
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Nonfat Dry Milk (weight to volume [w/v])

Blocking Buffer:
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix.
Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

Primary Antibody Dilution Buffer:
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA

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Bovine Serum Albumin (BSA)

Blotting Membrane: This protocol has been optimized for nitrocellulosemembranes, which CST recommends. PVDF membranes may also be used.

Sample Preparation

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape cells from plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose (or PVDF) membrane.

Flow Cytometry Protocol

A Solutions and Reagents

1. 1X Phosphate Buffered Saline (PBS): Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na2HPO4 and 0.24 g KH2PO4 in 800 mL distilled water (dH2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.

2. Formaldehyde (methanol free)

3. Incubation Buffer: Dissolve 0.5 g bovine serum albumin (BSA) in 100mL 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at –20°C in 90% methanol.

and mix well. While stirring, add 20 µl Tween-20 (100%).

Membrane Blocking and Antibody Incubations

Note: Volumes for 10 cm x 10 cm (100 cm2) membrane; for different sized membranes, adjust vol. accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.
8. Process membranes using enhanced chemiluminescence.

D Staining Using Unlabeled Primary and Conjugated Secondary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 0.5-1x106 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add the primary antibody at the appropriate dilution to the assay tubes (see individual antibody data sheet for the appropriate dilution).
6. Incubate for 30-60 minutes at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in fluorochrome-conjugated secondary antibody*, diluted in Incubation Buffer according to the manufacturer's recommendations.
9. Incubate for 30 minutes at room temperature.
10. Rinse as before in Incubation Buffer by centrifugation.
11. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.

*Recommended Secondary Antibodies from Invitrogen.
A-11070 Alexa Fluor® 488 F(ab')2 fragment of goat anti-rabbit IgG (H+L) (1:1000 dilution)
A-11017 Alexa Fluor® 488 F(ab')2 fragment of goat anti-mouse IgG (H+L) (1:1000 dilution)

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