Anti–COX-1/Anti–COX-2

Catalog No. 334090  50 Tests  20 µL/test

RESEARCH APPLICATIONS

Research applications include studies of:

- Intracellular detection of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) proteins
- Anti-inflammatory drug development (coxibs)

DESCRIPTION

Specificity

Anti–COX-1 recognizes a homodimeric, heme-containing, glycosylated, constitutively expressed enzyme that is located on the luminal surface of the endoplasmic reticulum and on the inner and outer membranes of the nuclear envelope. Anti–COX-2 recognizes the inducible form of this enzyme.

Antigen distribution

Cyclooxygenases are pivotal enzymes in cellular prostaglandin biosynthetic pathways and catalyze reactions in which arachidonic acid is converted to the endoperoxide intermediate, prostaglandin H2. There are two enzymatically active isoforms of the cyclooxygenase enzyme, COX-1 and COX-2. Both isoforms have similar biological functions and share approximately 60% primary sequence identity. Both enzymes are located on the luminal surface of the endoplasmic reticulum and on the inner and outer membranes of the nuclear envelope. COX-1 is constitutively expressed in nearly all mammalian tissues and is responsible for the basal prostaglandin synthesis required for cell homeostasis. COX-2 expression is not detected in most tissues under physiological conditions but is inducible by various stimuli. The expression of COX-2 is rapidly upregulated during the course of inflammation, following cellular stresses, and in response to growth factors, tumor promoters, hormones, bacterial endotoxins, and inflammatory cytokines. COX-2 can be induced in a number of cell types, including fibroblasts, endothelial cells, monocytes, and ovarian follicles. Changes in cellular cyclooxygenase expression can be detected by intracellular staining of COX-1 and COX-2.

Clones

Anti–COX-1, clone AS70, is an antibody raised against human recombinant COX-1 protein. The antibody does not cross-react with human recombinant COX-2 by ELISA.

Anti–COX-2, clone AS67, is an antibody raised against human recombinant COX-2 protein. The antibody does not cross-react with human recombinant COX-1 by ELISA.

Composition

The Anti–COX-1 and Anti–COX-2 antibodies are each composed of mouse IgG1 heavy chains and kappa light chains.

The Anti–COX-1/Anti–COX-2™ reagent is supplied as a combination of Anti–COX-1 FITC and Anti–COX-2 PE in 1 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA), beta-lactoglobulin, and 0.1% sodium azide.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.
PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Lipopolysaccharide (LPS) activation of human whole blood monocytes induces intracellular COX-2 expression and can serve as a model system to study enzyme upregulation. COX-1 expression is not induced by LPS in human monocytes. Certain test samples will not require prior activation to detect intracellular expression of COX-1 or COX-2. A description of the LPS model system follows.7

Endotoxin Activation of Whole Blood Monocytes

1. Collect whole blood in a heparin BD Vacutainer™ tube (Catalog No. 366481).
2. To rapidly induce the upregulation of COX-2, transfer 1-mL aliquots of fresh human whole blood into Falcon® 15-mL polypropylene tubes (Catalog No. 352096).
3. Add LPS, 1.0 to 5.0 µg/mL of whole blood, as a stimulus.
4. Incubate the samples for 4 hours in a 37°C incubator with 7% CO₂.
   Use an unstimulated sample (no LPS) as a negative control.

Intracellular Immunofluorescent Staining of Endotoxin-Activated Whole Blood Monocytes

1. After incubation with LPS, transfer 100-µL aliquots of whole blood to 12 x 75-mm polystyrene tubes (Catalog No. 352052).
2. Stain with 20 µL of CD14 PerCP reagent (Catalog No. 340585) for 15 minutes in the dark at room temperature.
3. Treat samples with 2 mL of 1X BD FACS™ Lysing Solution (Catalog No. 349202) for 10 minutes in the dark at room temperature.
4. Centrifuge for 5 minutes at 500 x g; remove and discard the supernatant.
5. Permeabilize samples by incubating in the presence of 0.5 mL of 1X BD FACS Lysing Solution containing 0.2% saponin for 10 minutes in the dark at room temperature.
6. Wash samples by adding 2 mL of 1X PBS containing 1% BSA and 0.1% sodium azide and centrifuge as described in step 4.
7. Remove and discard the supernatant.
9. Incubate for 30 minutes in the dark at room temperature.
10. Wash with 2 mL of 1X PBS containing 1% BSA and 0.1% sodium azide.
11. Centrifuge 5 minutes at 500 x g; remove and discard the supernatant.
12. Resuspend samples to a final volume of 400 µL in 1% paraformaldehyde (PFA, in 1X PBS, Electron Microscopy Sciences, Fort Washington, PA, 10% EM Grade).

Store samples at 4°C until ready to analyze on a flow cytometer. We recommend analyzing within 24 hours.

NOTE  COX-2 expression levels in donors can vary over time in response to LPS stimulation.

NOTE  Cultured cell lines also can be stained by this method. LPS activation and CD14 staining might not be necessary.
**REPRESENTATIVE DATA**

Performed on a BD FACS™ brand flow cytometer.

In Figure 1 the dashed line represents the isotype control.

**Figure 1** Intracellular Anti–COX-1 (left) and Anti–COX-2 (right) staining of a fibroblastic cell line, CCD1070sk (ATCC# CRL-2091)

![Figure 1](image)

**Figure 2** LPS-unactivated and LPS-activated CD14+ whole blood monocytes (R1) intracellularly stained with Anti–COX-1/Anti–COX-2

![Figure 2](image)

**HANDLING AND STORAGE**

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

**WARNING**

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

**CHARACTERIZATION**

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

**WARRANTY**

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label or in the product labeling at the time of delivery to the customer. BD disclaims hereby all other warranties, expressed or implied, including warranties of merchantability and fitness for any particular purpose and noninfringement. BD's sole liability is limited to either replacement of the products or refund of the purchase price. BD is not liable for property damage or any incidental or consequential damages, including personal injury, or economic loss, caused by the product.

**REFERENCES**


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