

**DESCRIPTION**

**Source** *Spodoptera frugiperda*, Sf 21 (baculovirus)-derived human COX-2 protein  
Ala18-Leu604  
Accession # P35354.2  
with a C-terminal 6-His tag

**N-terminal Sequence Analysis** Ala18

**Predicted Molecular Mass** 68 kDa

**SPECIFICATIONS**

**SDS-PAGE** 65-71 kDa, under reducing conditions.

**Activity** Measured by its ability to convert arachidonic acid to prostaglandin H2.

**Endotoxin Level** <1.0 EU per 1 µg of the protein by the LAL method.

**Purity** >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Supplied as a 0.2 µm filtered solution in Tris, NaCl, CHAPS and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer A: 50 mM Tris, 5 µM Hemin (Sigma, Catalog # H9039), pH 8.0
  - Assay Buffer B: 50 mM Tris, pH 8.0
  - Recombinant Human COX-2 (rhCOX-2) (Catalog # 10465-CX)
  - Substrate Component 1: Arachidonic acid (Sigma, Catalog # 10931), 10 mM stock in DMSO
  - Substrate Component 2: Amplex Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 10 mM stock in DMSO
  - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
  - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhCOX-2 to 5 ng/µL in Assay Buffer A.
  2. Prepare Substrate Mixture 50 µM Arachidonic acid and 100 µM AUR in Assay Buffer B.
  3. Load into a plate 50 µL of diluted rhCOX-2. Also create a Substrate Blank by loading 50 µL of Assay Buffer A.
  4. Add 50 µL of Substrate Mixture to all wells and incubate at room temperature for 1 minute.
  5. Read at excitation and emission wavelengths of 540 nm and 585 nm (top read), respectively in endpoint mode.
  6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted Fluorescence* (RFU)} \times \text{Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$$

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard Resorufin (Sigma, Catalog # R3257)

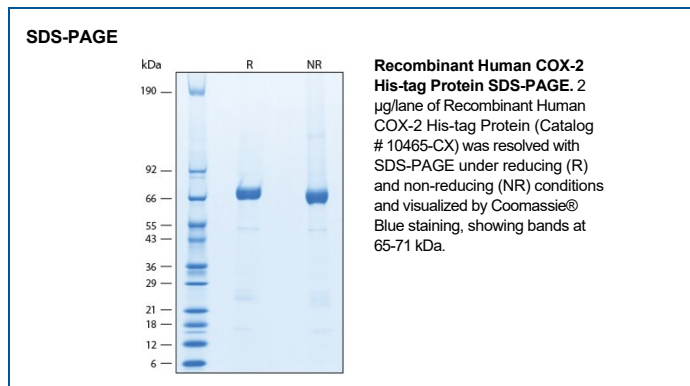
- Final Assay Conditions**
- Per Well
- rhCOX-2: 0.25 µg
  - Arachidonic acid: 25 µM
  - AUR: 50 µM

**PREPARATION AND STORAGE**

**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

- Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
  - 3 months, -20 to -70 °C under sterile conditions after opening.

**DATA**



**BACKGROUND**

Cyclooxygenase 2 (COX-2), also known as Prostaglandin synthase 2 (PTGS2), is a dimeric, heme-dependent enzyme where the dimer is formed from two monomers each containing an N-terminal EGF-like domain, a membrane binding domain and a C-terminal catalytic domain (1). COX-2 catalyzes the conversion of arachidonate to prostaglandin H2 through a two-step reaction, representing the rate-limiting enzyme in the biosynthesis of prostanoids. Altered levels of prostaglandins are associated with several disease pathologies. There are two major isozymes of PTGS with slightly differing substrate preferences; PTGS1 represents the constitutively expressed form while PTGS2 basal expression is present in a limited number of tissues (2) such as inflammatory cells. PTGS2 is upregulated in chronic and acute inflammation as well as in most types of cancers (3) resulting in increased rate of recurrence (4), reduction in survival (5), and increased resistance to chemo and radiotherapy (6) through the role it plays in multiple pathways and mechanisms (7). Inhibition of PTGS2 is a promising therapeutic target to reduce cancer risk (8, 9). COX-2 is a pharmacological target of nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors (coxibs) as well as other categories of inhibitors through multiple mechanisms of action (1). It remains an attractive target for novel specific inhibitor development as inhibition shows equivalent efficacy to use of conventional NSAIDs, but with reduced negative side effects (10, 11).

**References:**

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