

## ORDERING INFORMATION

**Catalog Number:** AF3170

**Lot Number:** WUM01

**Size:** 100 µg

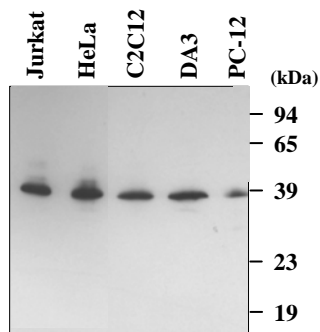
**Storage:** -20° C

**Specificity:** human/mouse/rat HO-2/HMOX2

**Immunogen:** *E. coli* derived recombinant human HO-2 (aa 1 - 316)

**Ig Type:** goat IgG

**Application:** Western blot



## Detection of HO-2/HMOX2 with AF3170.

Lysates from human Jurkat and HeLa, mouse C2C12, and DA3 and rat PC-12 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.5 µg/mL goat anti-HO-2 as described in *Protocols for Immunoblotting*. A 15 second exposure to film is shown.

## Background

Heme Oxygenase 2 (HO-2), also known as HMOX2, is a 36 kDa microsomal enzyme required for the metabolism of heme to biliverdin. Heme oxygenase occurs as 2 isozymes, the constitutively expressed heme oxygenase-2 (HO-2/HMOX2) and the inducible heme oxygenase-1 (HO-1/HMOX1). HO-1 expression is induced by heme and other non-heme compounds. Human HO-2 shares 42% amino acid sequence identity with human HO-1 and 89% amino acid sequence identity with mouse and rat HO-2.

## Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Heme Oxygenase 2 (rhHO-2; aa 1 - 316; Accession # NP\_002125). Polyclonal antibody was affinity-purified on a column derivatized with recombinant human HO-2.

## Formulation

Lyophilized from a 0.2 µm-filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute the antibody in 100 µL PBS containing 0.02% NaN<sub>3</sub>. The antibody concentration will be 1.0 mg/mL.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity.

**Avoid repeated freeze-thaw cycles.**

## Specificity

The antibody is known to react with endogenous human, mouse, and rat HO-2 on Western blots.

## Applications

**Western blot** - An antibody concentration of 0.5 µg/mL is recommended.

**Optimal dilutions should be determined by each laboratory for each application.**

## ***Protocols for Immunoblotting:***

### **Western blotting**

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	5% nonfat dry milk in Blotting Buffer	2% nonfat dry milk in Blotting Buffer
0.15 M NaCl		
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in antibody solution containing 0.5 µg/mL goat anti-human/mouse/rat HO-2.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.
6. Detect with Western Glo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

**Cell lysates for Western blots:** To prepare total cell lysates, cells are solubilized in hot 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3 - 4 bursts of 5-10 second each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

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