

ORDERING INFORMATION

Catalog Number: AF3776

Lot Number: YAU01

Size: 100 µg

Storage: -20° C

Specificity: human/mouse HO-1/HMOX1

Immunogen: *E. coli* derived recombinant human HO-1/HMOX1 (aa 1-261)

Ig Type: goat IgG

Application: Western blot

Background

Heme Oxygenase 1 (HO-1), also known as HMOX1 and Heat Shock Protein 32 (HSP32), is a 32 kDa microsomal enzyme required for the metabolism of heme to biliverdin. Heme oxygenase occurs as 2 isozymes, an inducible heme oxygenase-1 (HO-1/HMOX1) and a constitutive heme oxygenase-2 (HO-2/HMOX2). HO-1 expression is induced by heme and other non-heme compounds. Human HO-1 shares 82% amino acid sequence identity with mouse HO-1.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human Heme Oxygenase 1 (rhHO-1; aa 1 - 261; Accession # P09601). Polyclonal antibody was affinity-purified on a column derivatized with recombinant human HO-1.

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% NaN₃. 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.

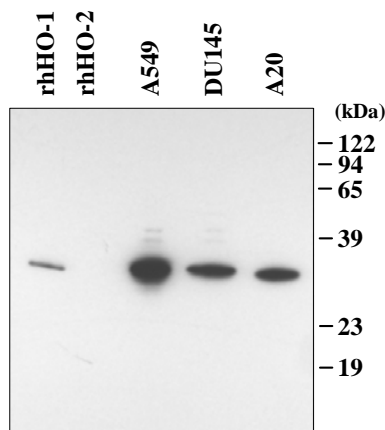
Specificity

The antibody detects endogenous human and mouse HO-1 on Western blots. Goat anti-human/mouse HO-1 does not detect recombinant human HO-2 on Western blot.

Application

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

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



Detection of HO-1/HMOX1 with AF3776.

Lysates from human A549 and DU145, and mouse A20 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 0.5 µg/mL goat anti-HO-1 as described in *Protocols for Immunoblotting*. A 15 second exposure to film is shown.

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 HO-1.
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 rabbit anti-goat IgG (R&D Systems, Catalog # HAF017).
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 2×10^6 - 1×10^7 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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