

## ORDERING INFORMATION

**Catalog Number:** MAB4344

**Clone:** 453709

**Lot Number:** CAJV02

**Size:** 100 µg

**Storage:** -20° C

**Specificity:** human PON2

**Immunogen:** *E. coli*-derived rhPON2

**Ig Class:** rat IgG<sub>2A</sub>

**Recommended Application:**  
Western blot

## Background

The paraoxonase (PON) gene family of antioxidant enzymes includes three known members located adjacent to each other on chromosome 7. Paraoxonase/arylesterase 2 (PON2) is a 354 amino acid, 39 kDa protein that is widely expressed in a variety of tissues and may act as a cellular antioxidant, protecting cells from oxidative stress. PON2 has arylesterase and aryldialkylphosphatase activity (EC 3.1.1.2 and EC 3.1.8.1) and can hydrolyze a number of organophosphate substrates and aromatic carboxylic acid esters. PON2 is membrane-bound and has several potential glycosylation sites. Sequence polymorphisms in this gene may be associated with coronary heart disease and a number of phenotypes related to diabetes. PON2 is not associated with HDL but can prevent LDL lipid peroxidation and reverse the oxidation of mildly oxidized LDL. Alternatively spliced transcript variants encoding different isoforms have been described.

## Preparation

This antibody was produced using a hybridoma elicited from a rat immunized with purified, *E. coli*-derived recombinant human PON2 (rhPON2; aa 30 - 354; Accession # Q16165). The IgG fraction of the hybridoma culture supernatant was purified by protein G chromatography.

## Formulation

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

## Reconstitution

Reconstitute in 100 µL of PBS containing 0.02% NaN<sub>3</sub>.

## 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 PON2 at ~40-42 kDa by Western blot. This antibody does not cross react with rhPON1 or rhPON3 on Western blot.

## Application

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

### Protocols for Immunoblotting

#### Blotting Buffer

25 mM Tris, pH 7.4  
0.15 M NaCl  
0.1% Tween® 20

#### Blocking Solution

5% nonfat dry milk  
in Blotting Buffer  
Adjust pH to 7.4

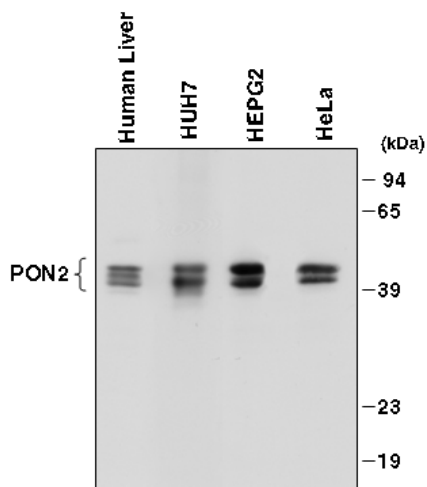
#### Antibody Solution

2% nonfat dry milk  
in Blotting Buffer  
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon 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 MAB4344.
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 at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated anti-rat IgG (R&D Systems, Catalog # HAF005).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent substrate detection system according to manufacturers protocol.

**Cell lysates for Western blottings** - To prepare total cell lysates, cells are solubilized in 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  $2 \times 10^5$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

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



### Detection of PON2 with MAB4344.

Lysates from human liver tissue and HUH-7, HepG2 and HeLa cells were resolved by SDS-PAGE, transferred to Immobilon membranes and immunoblotted with 0.5 µg/mL MAB4344, as described in *Protocols for Immunoblotting*.