

# Human Paraoxonase-1 Mouse Monoclonal Antibody (Clone KS-5F5)

Cat# CY-M1034

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
KS-5F5	WB, IP	H	37-44 kDa	Mouse IgG2b

## Background

Paraoxonase (PON, arylalkylphosphatase, EC 3.1.8.1) is a calcium-dependent esterase that is a component of plasma high density lipoproteins (HDLs), and hydrolyzes organophosphates (e.g., paraoxon, diazoxon) and arylesters (e.g., phenyl acetate) (1). Although its natural substrate is not certain, studies have indicated that PON hydrolyzes phospholipid peroxides in both HDLs and low density lipoproteins (LDLs) (2), and that this is one mechanism by which HDLs prevent the oxidative modification of LDLs (3). Two major genetic polymorphisms of PON have been described, due to glutamine or arginine at position 192 and methionine or leucine at position 55 (4). The paraoxonase activity of the Gln-192 (Q allele) isoform has been reported to be lower than that of the Arg-192 (R allele) isoform (5). Nevertheless, PON in QQ genotype subjects appears to be more effective than that in RR genotype subjects in protecting LDLs from oxidation (6). It has been suggested that the active site in PON that protects LDLs differs from the active sites for its paraoxonase and arylesterase activities (7). Low serum paraoxonase activity has been observed in patients with myocardial infarction (8), hypercholesterolemia (9), and diabetes mellitus (9), and there is evidence that the position 192 polymorphism may be a risk factor for coronary heart disease (CHD) in some populations (10). Such findings have indicated that PON plays an important role in lipid metabolism.

**Specificity/Sensitivity:** Human paraoxonase-1 Monoclonal Antibody detects endogenous paraoxonase-1 by western blotting and immunoprecipitation.

**Source/Purification:** Monoclonal antibody is produced by immunizing mice with a recombinant full length human paraoxonase-1. IgG is purified by protein A-sepharose chromatography.

**Recommended Antibody Dilutions:** Western blotting: 0.5-1 µg/mL, immunoprecipitation: 1-2 µg/sample.

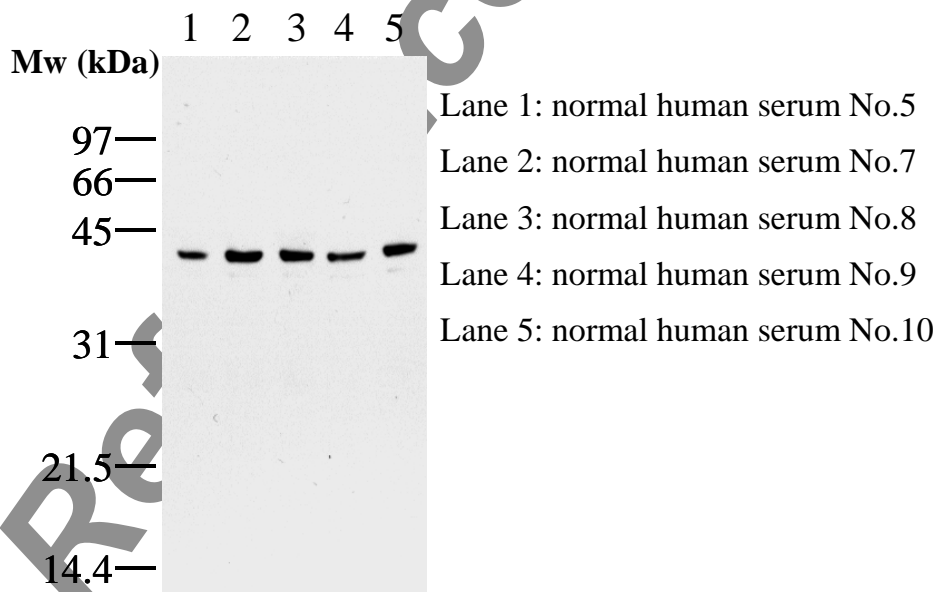
**Storage:** Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

**Applications Key:** WB: Western IP: Immunoprecipitation IHC: Immunohistochemistry IC: Immunocytochemistry F: Flow cytometry E: ELISA FP: Fluorescence Polarization assay

**Species Cross-Reactivity Key:** H: human M: mouse R: rat Hm: hamster Mk: monkey Mi: mink C: chicken X: *Xenopus* Z: zebra fish All: all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

**General References:**

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2. Aviram, M., M. Ronsenblat, C. L. Bisgaier, R. S. Newton, S. L. Primo-Parmo, and B. N. La Du.; Paraoxonase inhibits highdensity lipoprotein and preserves its functions: a possible peroxidative role for paraoxonase. J. Clin. Invest. 101: 1581-1590, 1998.
3. Mackness, M. I., S. Arrol, C. A. Abbott, and P.N. Durrington.; Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis. 104: 129-135, 1993
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6. Mackness, B., M. I. Mackness, S. Arrol, W. Turkie, and P. N. Durrington.; Effect of the human serum paraoxonase 55 and 192 genetic polymorphism on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett. 423: 57-60, 1998.
7. Aviram, M., S. Billecke, R. Sorenson, C. Bisgaier, R. Newton, M. Rosenblat, J. Eroglu, C. Hsu, C. Dunlop, and B. N. La Du.; Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities. Arterioscler. Thromb. Vasc. Biol. 18: 1617-1624, 1998.
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10. Serratto, M., and A. J. Marian.; A variant of human paraoxonase/ arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J. Clin. Chem. 96: 3005-3008, 1995.

**Fig.1 Western blot analysis of Human Paraoxonase-1**

## Western Immunoblotting Protocol

### Solutions and Reagents

*Note: Prepare solutions with Milli-Q or equivalently purified water.*

**Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

**SDS Sample Buffer (1X):** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

**Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).

**10X TBS (Tris-buffered saline):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

**Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 mL water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).

**Chemiluminescent HRP Detection:** secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

**Wash Buffer TBS/T:** 1X TBS, 0.1% Tween-20

**Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

### Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm<sup>2</sup> plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

### Membrane Blocking and Antibody Incubations

*Note: Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.*

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

### Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.

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2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

**Immunoprecipitation Followed by Western Immunoblotting Protocol****Solutions and Reagents**

*Note: Prepare solutions with Milli-Q or equivalently purified water.*

**Cell Lysis Buffer (1X):** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 µg/ml Leupeptin

*Note: We recommend adding 1 mM PMSF before use.*

**Protein A Agarose Beads:** Add 5 ml of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)

**3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue,

**Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

**Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk. For 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).

**10X TBS (Tris-buffered saline):** For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).

**Primary Antibody Dilution Buffer:** 1X TBS, 0.05% Tween-20 with 5% nonfat dry milk. For 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 10 µl Tween-20 (100%).

**Wash Buffer TBS/T:** 1X TBS, 0.1% Tween-20

**Chemiluminescent HRP Detection:** secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

**Wash Buffer TBS/T:** 1X TBS, 0.1% Tween-20

**Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

**Preparing Cell Lysates**

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm<sup>2</sup>) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

**Immunoprecipitation**

1. Take 200 µl cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
2. Add Protein A Agarose Beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1-3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 2 times with 500 µl of 1X Cell Lysis Buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µl 3X SDS Sample Buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95-100°C for 2-5 minutes.
6. Load the sample (15-30 µl) on SDS-PAGE gel (12-15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

*CircuLex*<sup>TM</sup>

Human Paraoxonase-1 Mouse Monoclonal Antibody

Product Data Sheet

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