Version 25-09/25

User's Manual

Human Myeloperoxidase ELISA Kit

REF **DEIA2776**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the quantitative determination of human Myeloperoxidase (MPO) concentrations in cell culture supernates, cell lysates, serum, platelet-poor plasma, saliva, and urine

General Description

Myeloperoxidase (MPO) is a heme-containing enzyme belonging to the XPO subfamily of peroxidases. It is an abundant neutrophil and monocyte glycoprotein that catalyzes the hydrogen peroxide dependent formation of hypochlorus acid (HOCl) and other reactive species (1, 2). Reaction of these compounds with macromolecules results in the nitrosylation, chlorination, and oxidation of tyrosine residues, lipids, and cholesterol, and the intermolecular crosslinking of proteins and DNA (1, 3-8). MPO is synthesized as a preproprotein that is proteolytically processed to remove a 48 amino acid (aa) signal peptide, a 116 aa propeptide, the C-terminal serine, and a 6 aa internal peptide which generates separate 60 kDa heavy and 12 kDa light chains (9). Other post-translational modifications of MPO include insertion of a heme moiety, glycosylation, and phosphorylation of mannose residues (10, 11). Enzymatically active MPO is a disulfide-linked tetramer that contains two heme groups and two copies each of the heavy and light chains (10, 11). Alternate splicing results in two additional isoforms of MPO, one with a 32 aa insertion in the light chain, and another with a deletion of the signal sequence and 47 aa of the propeptide (9). Mature human MPO shares 87-88% aa sequence identity with canine, mouse, and rat MPO. It shares 71%, 56%, and 47% aa sequence identity with comparable regions of human eosinophil peroxidase, lactoperoxidase, and thyroid peroxidase, respectively. MPO binds albumin, the macrophage mannose receptor, cytokeratin 1 on vascular endothelial cells, high molecular weight kininogen, and the integrin CD11b/CD18 on neutrophils (12-15). These interactions promote MPO clearance, a reduction of nitric oxide and bradykinin levels, reduced vasodilation, and continued neutrophil activation (12-16). MPO gene expression is under the control of thyroid hormone, retinoic acid, estrogen, and PPARγ receptors (17, 18). A polymorphism within the MPO gene promoter confers increased steroid responsiveness and is associated with the development or severity of a variety of diseases (1, 17, 18).

Neutrophil MPO is stored in cytoplasmic azurophilic granules (19, 20). Upon cellular activation and degranulation, MPO is delivered into phagosomes where it is required for the killing of phagocytosed bacteria (10, 21). Activated neutrophils also release granule contents extracellularly. Elevated plasma MPO levels have been associated with a variety of clinical conditions including systemic inflammation, eclampsia, risk of cardiovascular events, vascular endothelial dysfunction, severity of multiple sclerosis, and prospective mortality and oxidative stress during hemodialysis (22-30). MPO levels are also elevated in arthritic synovial fluid and gingivitis crevicular fluid (31, 32). MPO is incorporated into atherosclerotic plaques and renal stones and serves as an indicator of neutrophil infiltration of inflamed or injured tissues (33-35).

The Human MPO Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human MPO in cell culture supernates, cell lysates, serum, platelet-poor plasma, saliva, and urine.

Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MPO has been pre-coated onto a microplate. Standards and samples are pipetted into the

wells and any MPO present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MPO is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MPO bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. **Human MPO Microplate:** 1 plate, 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MPO.

   Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at ≤ -20°C*

2. **Human MPO Standard:** 2 vials, Natural human MPO in a buffered protein base with preservatives; lyophilized.

   Refer to the vial label for reconstitution volume.

   Use a new standard for each assay Discard after use.

3. **Human MPO Conjugate:** 2 vials, 11 mL/vial of a polyclonal antibody specifc for human MPO conjugated to horseradish peroxidase with preservatives.

   May be stored for up to 1 month at ≤ -20°C*

4. **Assay Diluent:** 2 vials, 6 mL/vial of a buffered protein base with preservatives.

   May be stored for up to 1 month at ≤ -20°C*

5. **Calibrator Diluent CD5K:** 2 vials, 11 mL/vial of a buffered protein base with preservatives.For cellculture supernate/cell lysate/saliva samples.

   May be stored for up to 1 month at ≤ -20°C*

6. **Calibrator Diluent CD6-58:** 2 vials, 11 mL/vial of a buffered protein base with preservatives. For serum/plasma/urine samples.

   May be stored for up to 1 month at ≤ -20°C*

7. **Wash Buffer Concentrate:** 2 vials, 11 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. May turn yellow over time.

   May be stored for up to 1 month at ≤ -20°C*

8. **Color Reagent A:** 1 vial, 12 mL of stabilized hydrogen peroxide.

   May be stored for up to 1 month at ≤ -20°C*

9. **Color Reagent B:** 1 vial, 12 mL of stabilized chromogen (tetramethylbenzidine).

   May be stored for up to 1 month at ≤ -20°C*

10. **Stop Solution:** 1 vial, 6 mL of 2N sulfuric add.

    May be stored for up to 1 month at ≤ -20°C*

11. **Plate Sealers:** 4 adhesive strips.

*Provided this is within the expiration date of the kit.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
2. Pipettes and pipette tips
3. Deionized or distilled water
4. Squirt bottle, manifold dispenser, or automated microplate washer
5. 500 mL graduated cylinder
6. Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
7. Test tubes for dilution of standards and samples
8. Human Myeloperoxidase Controls

If using cell lysate samples, the following is also required:

9. Cell Lysis Buffer
10. PBS

Storage

Store the unopened kit at ≤ -20 °C. Do not use past kit expiration date.

Specimen Collection And Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed before assaying. Refer to the Cell Lysis Procedure.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Platelet-poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

MPO is present in neutrophil granules and is released upon neutrophil exposure to activated platelets. Therefore, to measure circulating levels of MPO, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors released by platelet activation.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and

assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freezethaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples. 1. Perform a 5-fold dilution of Cell Lysis Buffer 3 with deionized or distilled water.

2. Wash cells one time in cold PBS.
3. Resuspend cells at 5 x 10^6 cells/mL in diluted Cell Lysis Buffer.
4. Incubate with gentle agitation for 30 minutes at room temperature and freeze/thaw cells once at ≤ -20 °C.
5. Centrifuge to remove cell debris.
6. Assay immediately or aliquot the lysis supernates and store at ≤ -20 °C until ready for use.

SAMPLE PREPARATION

Serum samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 90 µL of Calibrator Diluent CD6-58 followed by 50 µL of diluted sample + 200 µL of Calibrator Diluent CD6-58.

Platelet-poor plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent CD6-58.

Saliva samples require a 100-fold dilution. A suggested 100-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent CD5K followed by 20 µL of diluted sample + 180 µL of Calibrator Diluent CD5K.

Reagent Preparation

Bring all reagents to room temperature before use.

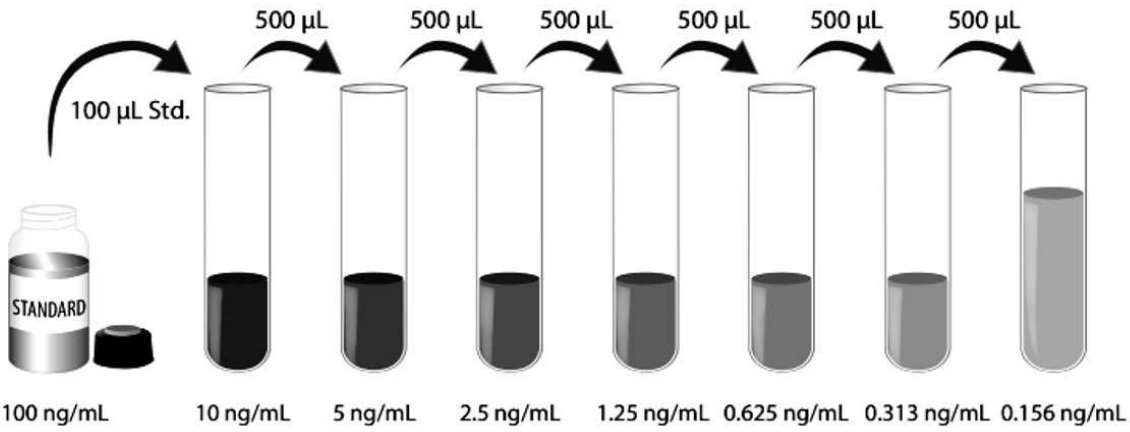
Note: MPO is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human MPO Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human MPO Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 900 µL of Calibrator Diluent CD5K (for cell culture supernate/cell lysate/saliva samples) or Calibrator Diluent CD6-58 (for serum/plasma/urine samples) into the 10 ng/mL tube.

Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL).



Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: MPO is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human MPO Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

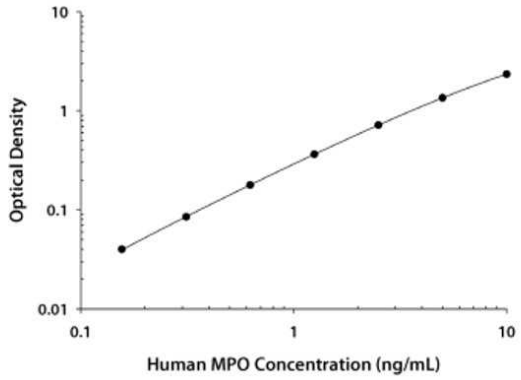
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human MPO concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

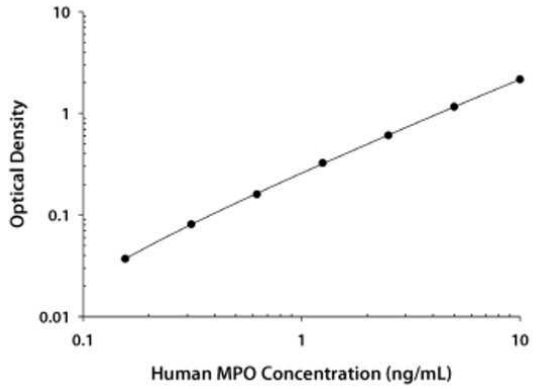
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/CELL LYSATE/SALIVA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	—
0.156	0.049 0.049	0.049	0.040
0.313	0.094 0.094	0.094	0.085
0.625	0.186 0.187	0.187	0.178
1.25	0.371 0.373	0.372	0.363
2.5	0.719 0.734	0.727	0.718
5	1.344 1.369	1.357	1.348
10	2.304 2.401	2.353	2.344

SERUM/PLASMA/URINE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.013 0.013	0.013	—
0.156	0.049 0.051	0.050	0.037
0.313	0.091 0.096	0.094	0.081
0.625	0.172 0.173	0.173	0.160
1.25	0.336 0.339	0.338	0.325
2.5	0.617 0.624	0.621	0.608
5	1.171 1.175	1.173	1.160
10	2.134 2.202	2.168	2.155

Performance Characteristics

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/CELL LYSATE/SALIVA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.994	3.36	6.68	0.974	2.99	6.23
Standard deviation	0.034	0.085	0.123	0.078	0.18	0.38
CV (%)	3.4	2.5	1.8	8.0	6.0	6.0

SERUM/PLASMA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	1.03	3.13	6.63	1.07	3.29	6.71
Standard deviation	0.024	0.046	0.172	0.12	0.26	0.55
CV (%)	2.3	1.5	2.6	10.8	8.0	8.2

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human MPO in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=29)	100	21.4-229	65
Platelet-poor EDTA plasma (n=29)	7.82	2.89-27.4	4.93
Platelet-poor heparin plasma (n=29)	9.47	3.57-28.4	5.27
Saliva (n=10)	558	31.2-1428	461
Urine (n=13)	4.39	0.17-13.5	4.68

Cell Culture Supernates:

Human monocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for human MPO.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	28.0	41.4
Stimulated	29.9	30.3

HL-60 human acute promyelocytic leukemia cells were cultured in RPMI supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human MPO, and measured 18.2 ng/mL.

An aliquot of the same HL-60 cells was removed, and the cells were lysed at 5 x 10^6 cells/mL in diluted Cell

Lysis Buffer. The lysate was assayed for human MPO and measured 2673 ng/mL.

Sensitivity

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of human MPO ranged from 0.003-0.062 ng/mL. The mean MDD was 0.014 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes natural human MPO.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range human MPO control were assayed for interference. No significant cross-reactivity or interference was observed.

1. **Recombinant human:**

   COX1

   Glutathione Peroxidase

   HMW Kininogen

   Integrin β2

   MMP-12

   Thyroid Peroxidase

2. **Recombinant mouse:**

   MPO

3. **Natural proteins:**

   bovine MPO

   human Neutrophil Elastase

   Natural human Eosinophil Peroxidase cross-reacts at approximately 1.8%.

Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human MPO were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=5)	Platelet-poor		Saliva* (n=4)	Urine* (n=4)
				EDTA plasma* (n=4)	Heparin plasma* (n=4)		
1:2	Average % of Expected	98	108	106	100	102	98
	Range (%)	96-100	100-114	102-108	93-105	99-107	97-98
1:4	Average % of Expected	100	108	109	104	102	98
	Range (%)	94-103	101-117	103-115	95-111	100-105	93-102
1:8	Average % of Expected	102	110	115	106	106	99
	Range (%)	96-107	98-120	113-117	95-114	103-111	94-104
1:16	Average % of Expected	99	108	112	103	97	92
	Range (%)	95-104	100-114	101-120	90-111	97-99	85-97

*Samples were diluted prior to assay.

Recovery

The recovery of human MPO spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	92-105%
Serum (n=4)	100	88-110%
Platelet-poor EDTA plasma (n=4)	95	90-101%
Platelet-poor heparin plasma (n=4)	91	83-100%

Precautions

1. MPO is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.
2. The Human MPO Standard provided in this kit was derived from human blood. The source material was tested at the donor level and found to be nonreactive for anti-HIV-1/2, anti-HCV, and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, the Human MPO Standard should be handled as if capable of transmitting infection.
3. The Stop Solution provided with this kit is an acid solution.
4. Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
5. Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
6. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. When using an automated plate washer, adding a 30 second soak period following the addition of Wash

Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
6. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Limitations

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
5. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. Variations in sample collection, processing, and storage may cause sample value differences.
7. This assay is designed to eliminate interference by enzymes, proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

References

1. Klebanoff, S.J. (2005) J. Leukoc. Biol. 77:598.
2. Morishita, K. et al. (1987) J. Biol. Chem. 262:3844.
3. van Dalen, C.J. et al. (2000) J. Biol. Chem. 275:11638.
4. Podrez, E.A. et al. (1999) J. Clin. Invest. 103:1547.
5. Bergt, C. et al. (2004) Proc. Natl. Acad. Sci. 101:13032.
6. Hazen, S.L. et al. (1996) J. Biol. Chem. 271:23080.
7. Heinecke, J.W. et al. (1993) J. Clin. Invest. 91:2866.
8. Kulcharyk, P.A. and J.W. Heinecke (2001) Biochemistry 40:3648.
9. Hashinaka, K. et al. (1988) Biochemistry 27:5906.
10. Hansson, M. et al. (2006) Arch. Biochem. Biophys. 445:214.
11. Nauseef, W.M. et al. (1992) Blood 80:2622.
12. Tiruppathi, C. et al. (2004) Proc. Natl. Acad. Sci. 101:7699.
13. Shepherd, V.L. et al. (1990) Am. J. Respir. Cell Mol. Biol. 2:335.
14. Astern, J.M. et al. (2007) Am. J. Pathol. 171:349.
15. Lau, D. et al. (2005) Proc. Natl. Acad. Sci. 102:431.
16. Eiserich, J.P. et al. (2002) Science 296:2391.
17. Kumar, A.P. et al. (2004) J. Biol. Chem. 279:8300.

18. Piedrafita, F.J. et al. (1996) J. Biol. Chem. 271:14412.
19. Cramer, E. et al. (1985) Blood 65:423.
20. Borregaard, N. and J.B. Cowland (1997) Blood 89:3503.
21. Hirche, T.O. et al. (2005) J. Immunol. 174:1557.
22. Sun, Y.C. et al. (2007) Clin. Chim. Acta 378:175.
23. Andelid, K. et al. (2007) Respir. Med. 101:888.
24. Noyan, T. et al. (2006) Aust. N.Z. J. Obstet. Gynaecol. 46:486.
25. Meuwese, M.C. et al. (2007) J. Am. Coll. Cardiol. 50:159.
26. Baldus, S. et al. (2003) Circulation 108:1440.
27. Vita, J.A. et al. (2004) Circulation 110:1134.
28. Minohara, M. et al. (2006) J. Neuroimmunol. 178:156.
29. Kalantar-Zadeh, K. et al. (2006) Am. J. Kidney Dis. 48:59.
30. Wu, C.C. et al. (2005) Nephrol. Dial. Transplant. 20:1134.
31. Steinbeck, M.J. et al. (2007) J. Orthop. Res. 25:1128.
32. Ortiz, G.C. et al. (1997) Eur. J. Oral Sci. 105:143.
33. Daugherty, A. et al. (1994) J. Clin. Invest. 94:437.
34. Mushtaq, S. et al. (2007) Clin. Chim. Acta 384:41.
35. Irazuzta, J.E. et al. (2000) Life Sci. 67:365.