Myeloperoxidase (human) ELISA Kit

Item No. 501410

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TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safety Data
4 Precautions
4 If You Have Problems
5 Storage and Stability
5 Materials Needed but Not Supplied

INTRODUCTION
6 Background
6 About This Assay
7 Principle Of This Assay
8 Definition of Key Terms

PRE-ASSAY PREPARATION
9 Buffer Preparation
9 Sample Preparation
10 Sample Matrix Properties

ASSAY PROTOCOL
14 Preparation of Assay-Specific Reagents
15 Plate Set Up
16 Performing the Assay

ANALYSIS
19 Calculations
20 Performance Characteristics

RESOURCES
23 Troubleshooting
25 Plate Template
26 References
27 Notes
27 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401412	Anti-MPO (human) ELISA Strip Plate	1 plate
400507	Anti-MPO (human) HRP Conjugate	1 vial/1.5 ml
401344	MPO (human) ELISA Standard	1 vial
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400074	TMB Substrate Solution	1 vial/12 ml
10011355	HRP Stop Solution	1 vial/12 ml
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Myeloperoxidase (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. An orbital microplate shaker set at ~500 rpm.
2. A plate reader capable of measuring absorbance at 450 nm.
3. Adjustable pipettes and a repeating pipettor.
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Myeloperoxidase (MPO) is a heme-containing peroxidase and the most abundant protein in polymorphonuclear leukocytes (PMNs).[1] It can oxidize a variety of substrates and catalyzes the formation of highly reactive (pseudo)hypohalous acids and radicals, including hypochlorous acid (HOCl), using hydrogen peroxide (H_2O_2) for chlorination or peroxidation.[1] The use of H_2O_2 by MPO for either its chlorination or peroxidation activities depends on the relative concentrations of chloride and the reducing substrate.[2] MPO also has roles in PMN apoptosis and antimicrobial defense systems, including neutrophil extracellular trap (NET) formation and NETosis.[1,3,4] It enhances neutrophil elastase-induced chromatin decondensation and produces reactive oxygen species (ROS), which trigger NET formation.[5] MPO-derived oxidants and chlorinated products are enriched in LDL and human atherosclerotic lesions.[6-8] In addition, MPO levels in leukocytes and the blood are elevated in patients with coronary artery disease (CAD), and elevated serum levels of MPO in patients with acute coronary syndromes are considered a risk factor for subsequent cardiovascular events.[7-9] Due to its roles in the innate immune response and various diseases, MPO is a potential therapeutic target.

About This Assay

Cayman's MPO (human) ELISA Kit is a sandwich assay that can be used to measure MPO in plasma and serum without prior sample purification. This assay has been validated using plasma and serum from healthy volunteers and the results shown to be consistent with published data. The assay has also been validated in RPMI-1640 with 10% fetal bovine serum, a standard tissue culture medium. The standard curve spans the range of 0-10 ng/ml.

Principle Of This Assay

This sandwich assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for MPO (mouse anti-human MPO). This antibody will bind any MPO introduced into the well. Standards and samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of an HRP-labeled MPO monoclonal antibody to detect the captured MPO. The two antibodies form a 'sandwich' by binding to different locations on the MPO protein. The concentration of the analyte is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound conjugate, which in turn is proportional to the concentration of the MPO.

$$\textbf{Absorbance} \propto \textbf{[Anti-MPO HRP]} \propto \textbf{[MPO]}$$

A schematic of this process is shown in Figure 1, on page 8.

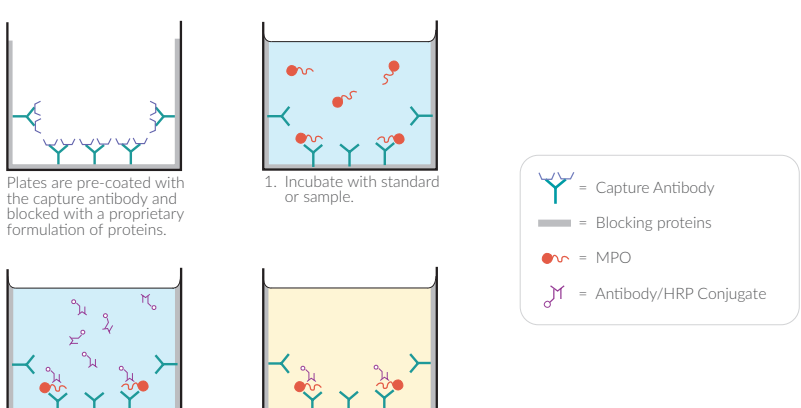


Figure 1. Schematic of the ELISA

Definition of Key Terms

LLOQ (Lower Limit of Quantification): the lowest standard concentration in which absorbance (450 nm) - (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **ELISA Buffer (1X) Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20. Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

In general, human serum, human plasma, and cell culture medium can be used directly in the assay following dilution in ELISA Buffer (1X). All plasma and serum samples must be diluted at least 1:5 in ELISA Buffer (1X). A minimum volume of 200 µl of each diluted sample is needed to run the sample in duplicate in the assay. For convenience, it is recommended to prepare 250 µl of each diluted sample. Dilutions should be performed using polypropylene tubes.

Sample Matrix Properties

Parallelism

To assess parallelism, human plasma, human serum, and cell culture supernatants from stimulated neutrophils were assayed at multiple dilutions using the MPO (human) ELISA Kit. Concentrations were plotted as a function of the sample dilution. The results are shown below.

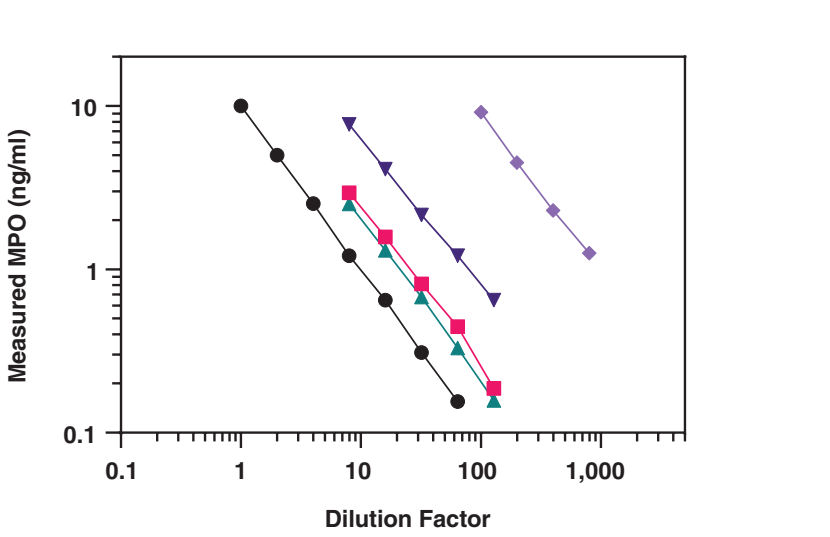


Figure 2. Parallelism of sample matrices

Linearity

Human plasma, human serum, and cell culture supernatants from stimulated human neutrophils with biologically relevant levels of MPO were tested at multiple dilutions using the MPO (human) ELISA Kit. The results are shown in the table below.

Dilution Factor	Measured MPO (ng/ml)	Linearity (%)
Heparin plasma, spiked with 340 ng/ml MPO		
20	312	100
40	351	113
80	385	123
EDTA plasma, unspiked		
8	20.2	100
16	20.9	103
32	21.7	107
64	21.2	105
128	20.2	100
Serum, unspiked		
8	61.7	100
16	65.6	106
32	68.8	112

Dilution Factor	Measured MPO (ng/ml)	Linearity (%)
Stimulated PMN supernatant, spiked with 400 ng/ml MPO		
100	1,294	100
200	1,288	100
400	1,264	98
800	1,302	101
Stimulated PMN supernatant, unspiked		
100	917	100
200	900	98
400	918	100
800	1,004	109

Table 1. Linearity of various matrices

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

Spike and Recovery

Human plasma (heparin) and cell culture medium (RPMI containing 10% FBS) were spiked with MPO, diluted as described in the **Sample Preparation** section, and analyzed using the MPO (human) ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

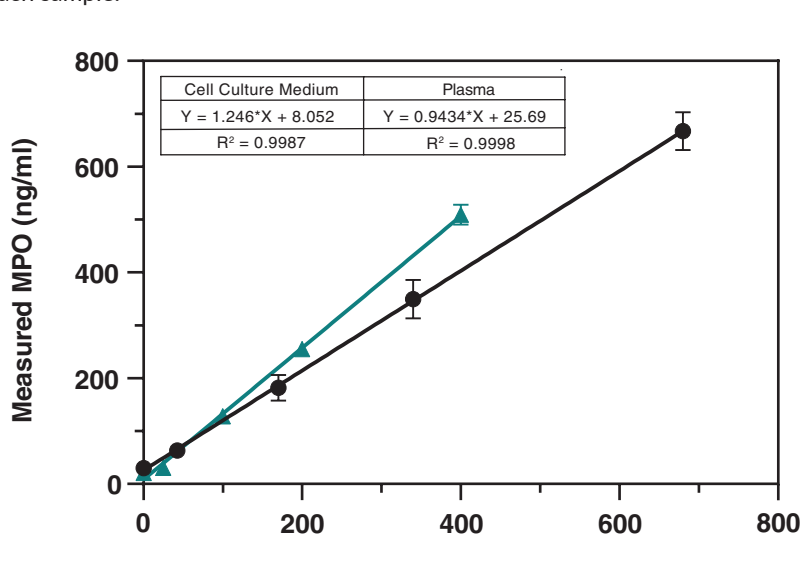


Figure 3. Spike and recovery

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

MPO (human) ELISA Standard

Reconstitute the lyophilized MPO (human) ELISA Standard (Item No. 401344) with 1 ml of ELISA Buffer (1X). Mix gently. The concentration of this solution (the bulk standard) is 100 ng/ml. The reconstituted standard will be stable for four weeks at 4°C.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes and label them #1 through #8. Aliquot 900 µl ELISA Buffer (1X) to tube #1 and 400 µl ELISA Buffer (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (100 ng/ml) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standard curve, will be 10 ng/ml. Serially dilute the standard by removing 400 µl from tube #1 and placing it in tube #2; mix thoroughly. Repeat this process for tubes #3-7. Do not add any MPO to tube #8. This tube is the background control.

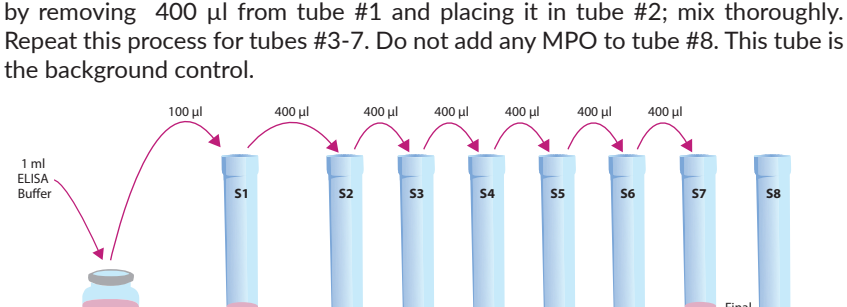


Figure 4. Preparation of the MPO standards

Anti-MPO (human) HRP Conjugate

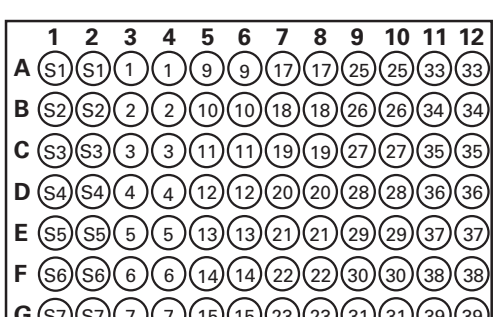
This reagent is supplied as a concentrated (10X) stock solution of anti-MPO (human) antibody conjugated to HRP. On the day of the assay, thaw the Anti-MPO (human) HRP Conjugate (Item No. 400507). For a full plate, dilute 1.2 ml of Anti-MPO (human) HRP Conjugate into 10.8 ml of ELISA Buffer (1X); for a half plate, dilute 0.6 ml of Anti-MPO (human) HRP Conjugate into 5.4 ml of ELISA Buffer (1X). Do not prepare the diluted HRP conjugate until immediately before use. Store unused Anti-MPO (human) HRP Conjugate at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, plate the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown Figure 5, on page 16. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest recording the contents of each well on the template sheet provided (see page 25).



S1-S8 - Standards 1-8
1-39 - Samples
Blk - Blank

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the standard, sample, and detection antibody.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of Standards and Samples and First Incubation

1. Pipette 100 µl of the MPO ELISA Standards into the appropriate wells on the plate.
2. Pipette 100 µl of diluted sample into each well. *NOTE: All samples must be diluted with ELISA Buffer (1X) according to the **Sample Preparation** section, see page 9.*
3. Cover the plate with the 96-Well Cover Sheet (Item No. 40012) and incubate for two hours at room temperature on an orbital shaker.

Addition of the Anti-MPO (human) HRP Conjugate and Second Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). Each well should be completely filled with wash buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer (1X).
2. Prepare a 1X working solution of the Anti-MPO (human) HRP Conjugate as described in the **Preparation of the Assay-Specific Reagents** section.
3. Add 100 µl of the Anti-MPO (human) HRP Conjugate working solution to each well of the plate.
4. Cover the plate with the 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). Each well should be completely filled with wash buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer (1X).
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-Well Plate Cover and incubate for 30 minutes at room temperature on an orbital shaker protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. If your plate reader is capable of analyzing the data, we recommend using a linear fit. Alternatively, a spreadsheet program can be used.

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a linear equation, or alternatively, a four-parameter logistic fit. Using the equation of the line, calculate the concentration of MPO in each sample.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You **must** run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

Absorbance (450 nm) at 30 minutes

MPO (human) ELISA Standard (ng/ml)	Absorbance (450 nm) Blank Adjusted	%CV Intra-Assay Precision	%CV Inter-Assay Precision
10	2.135	2.2	1.3
5	1.060	2.8	3.4
2.5	0.519	5.1	3.3
1.25	0.286	6.2	3.8
0.63	0.168	6.1	17.3
0.31	0.114	12.7	17.7
0.16	0.085	12.7	17.6
0	0.060	--	--

Table 2. Typical results

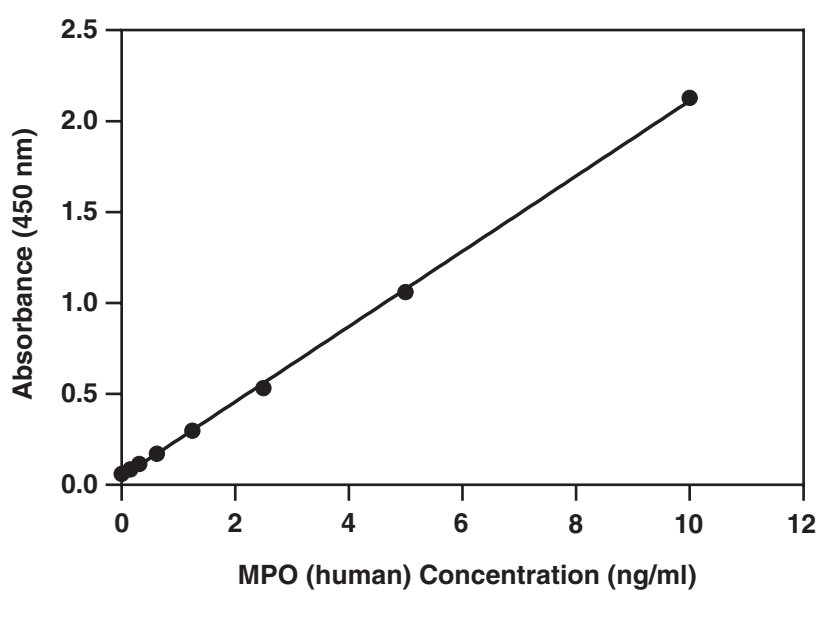


Figure 6. Typical standard curve

Precision

Intra-assay precision was determined by analyzing 24 replicates of three human plasma (EDTA) controls in a single assay.

Matrix Control (ng/ml)	%CV
331	17
178	14
72	13

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three human plasma (EDTA) controls in separate assays spanning across several days.

Matrix Control (ng/ml)	%CV
224	2.1
135	2.0
42	1.9

Table 4. Inter-assay precision

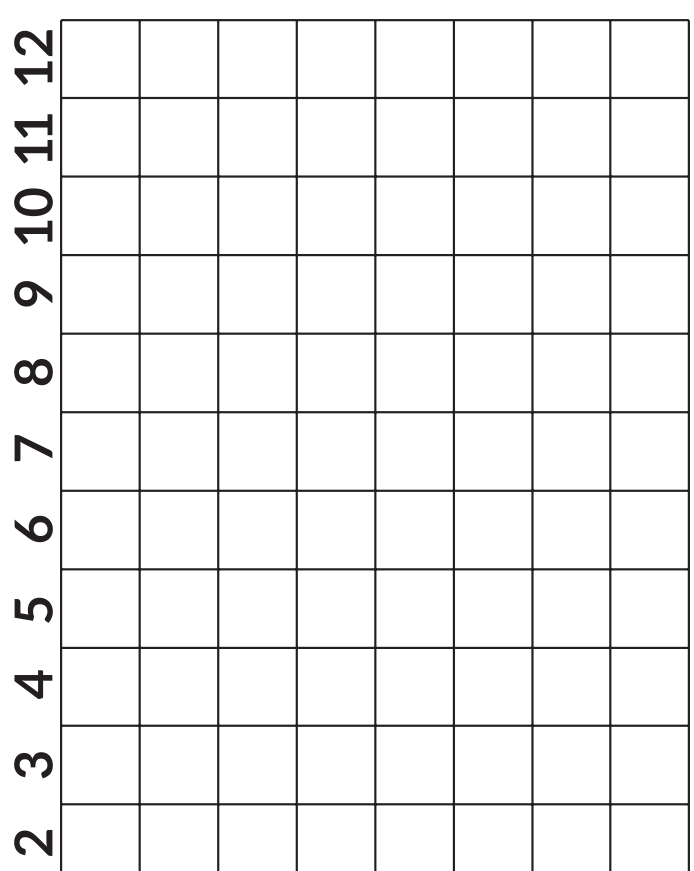
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of ultrapure water
Poor development (low signal) of standard curve	A. Standard is degraded B. 10X HRP conjugate was diluted into ELISA Buffer (1X) for too long before use, resulting in inactivation of HRP C. Standard was diluted incorrectly	Perform assay again with a new aliquot of standard or HRP conjugate
Sample concentrations appear inconsistent with literature values	Matrix for samples and standards are different	Use same matrix for all samples and standards

Procedure	Standards/Samples (µl)
Mix all reagents gently	--
Add standards/samples to plate	100
Seal the plate and tap gently to mix	
Incubate plate for 2 hours at RT, shaking	
Aspirate wells and wash 5 x well volume (~300 µl) with Wash Buffer (1X)	
Apply 1X HRP conjugate Solution	100
Incubate for 1 hour at RT, shaking	
Aspirate wells and wash 5 x well volume (~300 µl) with Wash Buffer (1X)	
Apply TMB Substrate Solution	100
Incubate 15-30 min at RT, shaking, sealed, and *protected from light*	
Do Not Wash, apply HRP Stop Solution	100
Read absorbance at 450 nm	

Table 5. Assay summary



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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.