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Mouse MPO ELISA Kit

Catalog No: CK210 **Size:** 1 x 96 tests

Specificity:	Mouse Myeloperoxidase (MPO)
Sensitivity:	600 pg/ml
Range:	0.61 to 150 ng/ml
Sample Type:	Cell supernatants, serum, plasma samples.

Introduction:

The Mouse MPO (Myeloperoxidase) ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse MPO in plasma (serum is not recommended in this assay due to mouse MPO being released from neutrophils into serum in the process of blood coagulation) and cell culture supernatants. This assay employs an antibody specific for mouse MPO coated on a 96-well plate. Standards and samples are pipetted into the wells and MPO present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse MPO antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MPO bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied in the kit:

Items	Quantity	Storage/Stability After Preparation
CK210-A Microplate coated with Anti-Mouse MPO: 12 strips x 8 wells	96 wells	1 month at 2-8°C
CK210-B Wash Buffer Concentrate (20x)	25 mL	1 month at 2-8°C
CK210-C Recombinant Mouse MPO Standards (1 vial is enough to run each standard in duplicate.)	2 vials	1 week at -80°C
CK210-D Assay Diluent C: Standard/Sample - Plasma Diluent Buffer	30 mL	n/a
CK210-E Assay Diluent B (5x): Standard/Sample/Cell Culture Medium Diluent	15 mL	1 month at 2-8°C
CK210-F Detection Antibody: Anti-Mouse MPO, Biotinylated Each vial is enough to coat ½ microplate.	2 vials	5 days at 2-8°C
CK210-G Streptavidin-HRP Concentrate (400x)	200 µl	Do not store and reuse.
CK210-H TMB One-Step Substrate Reagent (3, 3', 5, 5'-tetramethylbenzidine in buffered solution)	12 mL	n/a
CK210-I Stop Solution (0.2 M Sulfuric Acid) Caution: Acid, corrosive.	8 mL	n/a

Storage of Kit Reagents:
Stable for up to 1 year, unused, at -20°C, 2 years at -80°C or 6 months at 2-8°C. Opened Microplate Wells and reagents are stable for 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.
Avoid repeated freeze-thaw cycles.

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Materials/reagents required but not provided:
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer/software for data analysis
- Tubes to prepare standard or sample dilution

Preparation of Kit Reagents:
Bring all reagents and samples to room temperature (18-25°C) before use.

Sample Dilution
If your samples need to be diluted, Assay Diluent C (Item D) is used for dilution of plasma samples and 1x Assay Diluent B (Item E) is used for dilution of cell culture supernatants.

Suggested dilution for normal serum/plasma: 2-fold*.

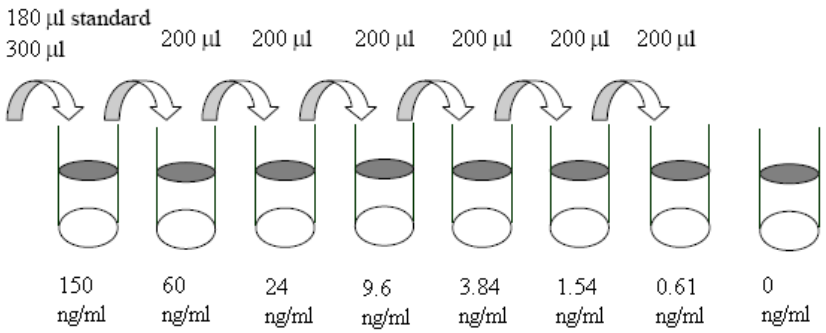
*Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Assay Diluent B
Dilute 5-fold with deionized or distilled water.

Wash Buffer Concentrate
- If the Wash Concentrate (Item B) contains visible crystals, warm to RT and mix gently until dissolved.
- Dilute 20 mL of Wash Buffer Concentrate into distilled water to yield 400 mL of 1x Wash Buffer.

MPO Standard
- Briefly spin the vial of Item C (Recombinant Mouse MPO Standard).
- Add 400 µl Assay Diluent C (for plasma samples) or 1x Assay Diluent B (for cell culture supernatants) to item C to prepare a 400 ng/mL standard.
- Dissolve the powder thoroughly by gentle mixing.
- Add 180 µl MPO standard (400 ng/ml) from the vial of Item C, into a tube with 300 µl Assay Diluent C or 1x Assay Diluent B to prepare a 150 ng/ml standard solution.
- Add 300 µl Assay Diluent C or 1x Assay Diluent B into each tube. Use the 150 ng/ml standard solution to produce a dilution series (shown below in Figure 1).
- Gently vortex to mix each tube thoroughly before the next transfer.
- Assay Diluent C or 1x Assay Diluent B serves as the zero standard (0 pg/mL).
- The 150 ng/ml standard point in Assay Diluent B may be saturated. We recommend starting with 60 ng/ml for the Assay Diluent B standard curve.

Figure 1



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Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Mix gently (the concentrate can be stored at 2-8°C for 5 days).
- Detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B for Step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and mix gently before use.
- Streptavidin-HRP concentrate should be diluted 400-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 30 µl of Streptavidin-HRP concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 400-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly.

ELISA Method:

Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay.

1. Bring all reagents and samples to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl of each standard (see **Preparation of Kit Reagents: MPO Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each). Wash by filling each well using a multi-channel Pipette or auto-washer. 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.

4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution and repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution and repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at RT or overnight at 2-8°C.

⇩

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at RT.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at RT.

⇩

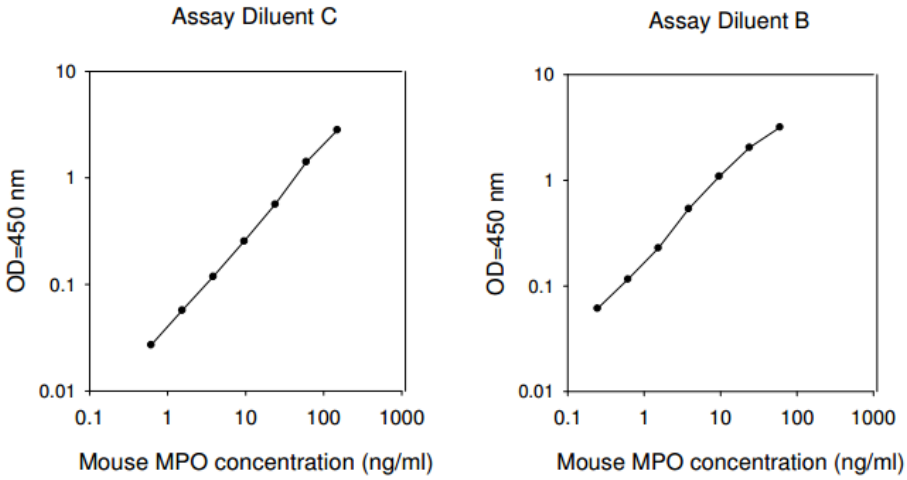
5. Add 100 µl TMB to each well.
   Incubate 30 minutes at RT.

⇩

6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately

Calculation of Results:
Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data:
Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



Performance and Characteristics:

Sensitivity
The minimum detectable dose of Mouse MPO was determined to be 600 pg/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is two standard deviations higher than that of the blank (diluent buffer).

Recovery

Recovery was determined by spiking various levels of Mouse MPO into mouse plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Plasma	86.89	68-105
Cell culture media	105.5	96-112

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Linearity

Sample Type		Plasma	Cell culture media
1:2	Average % of Expected	94.68	113.3
	Range (%)	83-106	105-120
1:4	Average % of Expected	123.5	114.7
	Range (%)	114-135	96-126

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

Specificity:

This ELISA pair antibody detects mouse MPO. Other species not yet determined.

Troubleshooting Guide:

Problem	Cause	Solution
1. Poor Standard Curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; change Assay Procedure Step 2 to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
	2. Air bubbles in wells	2. Remove bubbles from wells
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated Wash Buffer	2. Make fresh Wash Buffer
5. Low sensitivity	1. Improper storage of the ELISA kit	1. Store the Standard at -80°C after reconstitution. Store other reagents at 2-8°C. Keep Substrate Solution protected from light.
	2. Stop Solution	2. Stop Solution should be added to each well before measure.

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