ab100611

Macrophage Inflammatory Protein 3 Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human IGFBP4 concentrations in serum, plasma, cell culture supernatant and urine

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

ab100611 Human Macrophage Inflammatory Protein 3 (MIP3) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Macrophage Inflammatory Protein 3 in serum, plasma, cell culture supernatants and urine.

This assay employs an antibody specific for human Macrophage Inflammatory Protein 3 coated on a 96-well plate. Standards and samples are pipetted into the wells and Macrophage Inflammatory Protein 3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Macrophage Inflammatory Protein 3 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 Macrophage Inflammatory Protein 3 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.
2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 100µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.

Add 100µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.

Add 100µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.

Add 100µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

Add 50µl Stop Solution to each well. Read at 450 nm immediately.
3. Kit Contents

- Macrophage Inflammatory Protein 3 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human Macrophage Inflammatory Protein 3.
- Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
- Standards (Item C): 2 vials, recombinant human Macrophage Inflammatory Protein 3.
- Assay Diluent A (Item D): 30 ml of animal serum with 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
- Detection Antibody Macrophage Inflammatory Protein 3 (Item F): 2 vials of biotinylated anti-human Macrophage Inflammatory Protein 3 (each vial is enough to assay half microplate).
- HRP-Streptavidin concentrate (Item G): 200 µl of 200x concentrated HRP-conjugated streptavidin.
- TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3’,5,5’-tetramethylbenzidine (TMB) in buffered solution.
- Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
4. Storage and Handling

ab100611 may be stored for up to 6 months at 2 to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at –80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

5. Additional Materials Required

- 1 Microplate reader capable of measuring absorbance at 450nm.
- 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 and software for ELISA data analysis.
- 8 Tubes to prepare standard or sample dilutions.
6. Preparation of Reagents

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants and urine.

   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.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.

4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine, Assay Diluent B should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 80 µl Macrophage Inflammatory Protein 3 standard from the vial of Item C, into a tube with 586.7 µl Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay
Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part 7 Assay Method.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 60 µl of HRP-Streptavidin concentrate into a tube with 12 µl 1x Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin.
7. Assay Method

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 Reagents step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

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

4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. 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.
8. Data Analysis

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.

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable dose of Macrophage Inflammatory Protein 3 is typically less than 7 pg/ml.
C. Recovery

Recovery was determined by spiking various levels of human Macrophage Inflammatory Protein 3 into human serum, plasma and cell culture media. Mean recoveries are as follows:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>92.78</td>
<td>82-105</td>
</tr>
<tr>
<td>Plasma</td>
<td>94.16</td>
<td>83-106</td>
</tr>
<tr>
<td>Cell culture media</td>
<td>93.48</td>
<td>81-105</td>
</tr>
</tbody>
</table>

D. Linearity

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Serum Average % of Expected</th>
<th>Plasma Average % of Expected</th>
<th>Cell Culture Media Average % of Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>Serum Range (%)</td>
<td>Plasma Range (%)</td>
<td>Cell Culture Media Range (%)</td>
</tr>
<tr>
<td>1:2</td>
<td>88</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>80-103</td>
<td>81-104</td>
<td>82-103</td>
</tr>
<tr>
<td>1:4</td>
<td>93</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>82-104</td>
<td>81-105</td>
<td>83-104</td>
</tr>
<tr>
<td>1:8</td>
<td>91</td>
<td>94</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>81-102</td>
<td>83-105</td>
<td>82-103</td>
</tr>
</tbody>
</table>
E. Reproducibility

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

9. Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, PARC, PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF).
## 10. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.</td>
</tr>
<tr>
<td>Low signal</td>
<td>Too brief incubation times</td>
<td>Ensure sufficient incubation time; assay procedure step 2 change to over night</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Large CV</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td>High background</td>
<td>Plate is insufficiently washed</td>
<td>Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store your standard at &lt; -20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light</td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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