Introduction

Mac-2 binding protein (Mac-2bp), known as 90K, is a highly N-glycosylated, secreted protein, identified as a ligand of Galectin-3. It is considered that through interaction with Galectin-3, Mac-2bp promotes homotypic cell-cell contact or regulates cell adhesion. And it has been reported that Mac-2bp levels in body have associations with various human cancers or several viral infectious diseases. This ELISA kit can measure concentration of Mac-2bp.

Principle

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tera Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the amount of Human Mac-2bp.

Measurement range

0.78 - 100 ng/mL

Intended use

For research use only, not for use in diagnostic procedures.

This kit’s assay kit is capable for assay of human Mac-2bp in serum, EDTA-plasma and cell culture media.

The guide line of dilution rate for serum and plasma samples is from 500 to 1,000-fold.

Kit component

1. Precocated plate:
2. Labeled antibody Conc.:
3. Standard:
4. EIA buffer
5. Solution for labeled antibody:
6. Chromogen : TMB solution
7. Stop solution
8. Wash buffer Conc.

Operation manual

1. Materials needed but not supplied:
   - Plate reader (450nm)
   - Micropipette and tip
   - Graduated cylinder and beaker
   - Refrigerator (as 4°C)
   - Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
2. Preparation
   1) Preparation of wash buffer: "8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of 8. Washing Buffer Conc. as room temperature and then, mix it gently and completely before use. Dilute 50 μL of "8, Wash buffer Conc." with 1,950 μL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
   2) Preparation of labeled antibody: "2. Labeled antibody Conc." is a concentrated (30X). Dilute "2. Labeled antibody Conc." with "5. Solution for Labeled antibody" in 30X according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody. (Example) In case you use one strip (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2. Labeled antibody Conc." with 870 μL of "5. Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL in each well.) This operation should be done just before applying labeled antibody. The remaining "2. Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
   3) Preparation of Standard
   Put just 0.5 μL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 200 μL human Mac-2bp Standard.
   4) Dilution of standard
   Prepare 8 tubes for dilution of "3. Standard". Put 230 μL of each of "4, EIA buffer" into the tube.
   Specify the following concentration of each tube.

   - Tube-1: 100 ng/mL
   - Tube-2: 50 ng/mL
   - Tube-3: 25 ng/mL
   - Tube-4: 12.5 ng/mL
   - Tube-5: 6.25 ng/mL
   - Tube-6: 3.13 ng/mL
   - Tube-7: 1.56 ng/mL
   - Tube-8: 0.78 ng/mL
   - Tube-9: 0 ng/mL (Test Sample Blank)

3. Measurement procedure

   All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

   1) Determine wells for test sample blank, test sample and diluted standard.
   2) Pipette 20 μL of 20-fold diluted serum or plasma from the tube of above first dilution and add it to 480 μL of "4, EIA buffer" in another tube, and mix them well.
   3) Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently.
   4) Pipette 100 μL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
   5) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
   6) Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

   4) Dilution of test sample
   Test samples should be diluted with "4, EIA buffer" suitably. Serum or plasma samples have to be diluted with "4, EIA buffer" accordingly. The recommended dilution for them is from 500 to 1,000-fold. In case of the absorbance of sample is over than the assay range, it is necessary to dilute it more.

   5) Stop solution
   Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

   6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
   7) Wash the plate with the prepared wash buffer and remove all liquid.
   8) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
   9) Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

   1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
   2) Determine wells for test sample blank, test sample and diluted standard.
   3) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
   4) Wash the plate with the prepared wash buffer and remove all liquid.
   5) Incubate the precoated plate for 30 minutes at room temperature (shielded).
   6) Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

   7) Wash the plate with the prepared wash buffer and remove all liquid.
   8) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
   9) Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

   SPECIAL ATTENTION

   1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
   2) Test samples should be diluted with "4, EIA buffer", suitably.
   3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminants of organic solvent may affect the measurement.
5) Use only wash buffer in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) “6, Chromogen” should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT
Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. On a semilogarithmic paper the concentration of unknown samples can be plotted against absorbance (y-axis, linear). Draw the best smooth curve through these points. Read the concentration for unknown samples from the standard curve.

Example of standard curve

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1.929</td>
</tr>
<tr>
<td>50</td>
<td>1.691</td>
</tr>
<tr>
<td>25</td>
<td>1.363</td>
</tr>
<tr>
<td>12.5</td>
<td>0.983</td>
</tr>
<tr>
<td>6.25</td>
<td>0.664</td>
</tr>
<tr>
<td>3.13</td>
<td>0.399</td>
</tr>
<tr>
<td>1.56</td>
<td>0.236</td>
</tr>
<tr>
<td>0.78</td>
<td>0.133</td>
</tr>
<tr>
<td>0 (Test Sample Blank)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS
1. Dilution linearity

2. Added Recovery Assay

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Additive Amount (ng/mL)</th>
<th>Theoretical Value (ng/mL)</th>
<th>Measured Value (ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Plasma (EDTA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x500)</td>
<td>12.5</td>
<td>17.52</td>
<td>16.98</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>11.27</td>
<td>10.34</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>8.15</td>
<td>7.28</td>
<td>90.4</td>
</tr>
<tr>
<td>Human Serum (x500)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>17.11</td>
<td>16.11</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>10.86</td>
<td>9.71</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>7.74</td>
<td>6.90</td>
<td>89.2</td>
</tr>
<tr>
<td>Medium with 10% FBS (x10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>12.5</td>
<td>11.16</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>6.25</td>
<td>5.33</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>3.13</td>
<td>2.77</td>
<td>88.5</td>
</tr>
</tbody>
</table>

Mean Value (ng/mL)  SD (ng/mL)  CV (%)  n
42.25  2.46  5.6  22
11.83  0.53  4.5  22
2.96  0.10  3.4  22

Mean Value (ng/mL)  SD (ng/mL)  CV (%)  n
39.38  3.21  8.2  7
10.88  0.66  6.1  7
2.85  0.11  3.8  7

5. Sensitivity
0.08 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING
1. All reagents shall be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”. “4, EIA buffer” or “8, Wash buffer Conc.” however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.

STORAGE AND THE TERM OF VALIDITY
Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

REFERENCE