**COMPONENTS**

<table>
<thead>
<tr>
<th>Kit Component</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>96-well plate precoated with anti-mouse ICAM-1 antibody</td>
<td>1 Plate</td>
</tr>
<tr>
<td>Protein Standard: Lyophilized recombinant mouse ICAM-1</td>
<td>2 tubes, 10 ng/tube</td>
</tr>
<tr>
<td>Sample Diluent Buffer</td>
<td>30 ml</td>
</tr>
<tr>
<td>Biotinylated Antibody (Anti-mouse ICAM-1)</td>
<td>130 μl (100x)</td>
</tr>
<tr>
<td>Antibody Diluent Buffer</td>
<td>12 ml</td>
</tr>
<tr>
<td>Avidin-Biotin-Peroxidase Complex (ABC) Solution</td>
<td>130 μl (100x)</td>
</tr>
<tr>
<td>ABC Diluent Buffer</td>
<td>12 ml</td>
</tr>
<tr>
<td>Tetramethylbenzidine (TMB) Color Developing Agent</td>
<td>10 ml</td>
</tr>
<tr>
<td>TMB Stop Solution</td>
<td>10 ml</td>
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</table>

**Washing Buffer (not provided): TBS or PBS**

0.01M TBS: Add 1.2g Tris, 8.5g NaCl, 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 900ml H₂O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

0.01M PBS: Add 8.5g sodium chloride, 1.4g Na₂HPO₄, and 0.2g NaH₂PO₄ to 900ml distilled H₂O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

**Storage**

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.

**BACKGROUND**

ICAM-1 (intercellular adhesion molecule-1) is a transmembrane glycoprotein of 532 amino acids, which has a molecular mass ranging from 90 to 110 kDa. ICAM-1 is a member of the immunoglobulin supergene family, and contains five extracellular immunoglobulin-like domains. It is expressed on various cells of both hematopoietic and non-hematopoietic cells, including endothelial cells, leucocytes other than basophilic granulocytes, T cells, B cells, fibroblasts, and cancer cells. As a member of adhesion molecules, ICAM-1 binds to two integrins belonging to the beta2 subfamily CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) on the surface of leucocytes. ICAM1 is a key molecule in immune-mediated and inflammatory processes and function as a co-stimulatory signal which is important for the trans-endothelial migration of leucocytes and the activation of T cells. During leucocyte trans-endothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups through SGEF and RHOG activation. Moreover, ICAM-1 has a crucial role in the induction of an immune response and is instrumental in migration of T cells into inflamed tissue. ICAM-1 on target cells leads to recruitment of the MHC-I proteins to the contact area and enhances presentation of cognate peptide MHC-I complex to cytotoxic T cells. ICAM-1 interacts with important factors involved in many kinds of human cancers. ICAM-1 is involved in transmembrane signal transduction in the regulatory process of cell proliferation through the mitogen-activated protein kinase (MAPK) pathway and eventually the AP-1 pathway. ICAM-1 can be induced under inflammatory condition by TNF-alpha in a process that involves IκK-beta.

In addition, inhibition of ICAM-1 expression on melanoma cells reduces the metastatic ability of the melanoma cells, indicating an important role of ICAM-1 in metastasis. The existence of a soluble variant of ICAM-1 in the circulation has been described, with elevated levels being reported in several diseases; higher levels being associated with liver metastases in gastric, colonic, gall bladder and pancreatic cancer, and with reduced survival in patients with malignant melanoma. In case of rhinovirus infection, ICAM-1 acts as a cellular receptor for the virus.

**REFERENCES**


**ELISA OVERVIEW**

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunoabsorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethylbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

**NOTES:**

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.
PROTOCOL

I. Plate Washing
Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1-2 minutes. Repeat this process two additional times for a total of three washes.

II. Preparation of Test Samples

Test Sample Processing
• Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation.
• Serum: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 x g for 10 min.
• Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 20 min at 2000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

Sample Dilution Guideline
Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

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<tbody>
<tr>
<td>100-1000 ng/ml</td>
<td>1:100</td>
<td>1 μl</td>
<td>99 μl</td>
</tr>
<tr>
<td>10-100 ng/ml</td>
<td>1:10</td>
<td>10 μl</td>
<td>90 μl</td>
</tr>
<tr>
<td>15-1000 pg/ml</td>
<td>1:40</td>
<td>25 μl</td>
<td>75 μl</td>
</tr>
<tr>
<td>156-10000 pg/ml</td>
<td>1:250</td>
<td>50 μl</td>
<td>50 μl</td>
</tr>
<tr>
<td>≤156 pg/ml</td>
<td>n/a</td>
<td>100 μl</td>
<td>n/a</td>
</tr>
</tbody>
</table>

If samples will be assayed within 24 hours, store at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

III. Preparation of Reagents

Reconstitution of the Standard
The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.
1. 10,000 pg/ml of mouse ICAM-1 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. 5000 pg/ml - 156 pg/ml of mouse ICAM-1 standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000 pg/ml ICAM-1 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Preparation of Biotinylated Antibody Working Solution
The solution should be prepared no more than 2 hours prior to the experiment.
1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

Preparation of the Avidin-Biotin-Peroxidase Complex (ABC) Working Solution
The solution should be prepared no more than 1 hour prior to the experiment.
1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

IV. ELISA

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. A standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of target protein amount in samples.
1. Aliquot 0.1 ml per well of the 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml mouse ICAM-1 standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Blank well). Add 0.1 ml of each properly diluted sample of rat sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “Sample Dilution Guideline” for details. We recommend that each mouse ICAM-1 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-mouse ICAM-1 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 μl of prepared TMB color developing agent into each well and incubate plate at 37°C for 7-10 min (shades of blue can be seen in the wells with the four most concentrated mouse ICAM-1 standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

V. Calculating Protein Concentration

• For all wells, determine O.D.450(Reading):
  
  
  O.D.450(Reading) = O.D.450(Blank)

• Plot the standard curve:
  
  Plot O.D.450(Reading) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See Figure 1 for a typical standard curve.

• The target protein concentration in samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.

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