Product Name: Human IL-6 ELISA Kit for cell and tissue lysates

Product Number: RAB0307
Lot Number: 07130141

Storage:
Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components:
1. Human IL-6 Antibody-coated ELISA Plate (Item A) - RABIL6A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human IL-6.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human IL-6 Protein Standard (Item C) - RABMIL6S-1VL
4. Biotinylated Human IL-6 Detection Antibody (Item F) - RABIL6F-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 5x Sample Diluent Buffer (Item D2) - RABDIL6-10ML
9. ELISA 5x Assay/Sample Diluent Buffer (Item E2) - RABELADE-15ML
10. 2X Cell Lysis Buffer (Item J) - RABLYSIS1-5ML

Assay/Sample Diluent Buffer dilution (Preparation, Step 2)
Sample Diluent Buffer (Item D2) and Assay/Sample Diluent Buffer (Item E2) should be diluted 5-fold with deionized or distilled water before use. Cell lysis buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

Sample Dilution (Preparation, Step 3)
Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1X Sample Diluent Buffer (Item D2). Generally we recommend a minimum of 1 mg of protein per 1 ml of original lysate solution, though more concentrated is better. We also recommend the addition of protease inhibitors (not included) to the lysis buffer prior to use.

* Please note that the levels of IL-6 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
Preparation of Standard
(Preparation, Step 4)

Briefly spin a vial of Item C. Add 500 µl 1X Sample Diluent Buffer (Item D2) into Item C vial to prepare a 12,000 pg/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µl IL-6 standard from the vial of Item C, into a tube with 440 µl Sample Diluent Buffer to prepare a 1,000 pg/ml stock standard solution. Pipette 400 µl 1X Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. 1X Sample Diluent Buffer serves as the zero standard (0 pg/ml).

<table>
<thead>
<tr>
<th>Diluent volume</th>
<th>std1</th>
<th>std2</th>
<th>std3</th>
<th>std4</th>
<th>std5</th>
<th>std6</th>
<th>std7</th>
<th>zero standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item C+ 500 µl</td>
<td>440 µl</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
<td>400 µl</td>
</tr>
<tr>
<td>Conc. 12,000 pg/ml</td>
<td>1000 pg/ml</td>
<td>333.3 pg/ml</td>
<td>111.1 pg/ml</td>
<td>37.04 pg/ml</td>
<td>12.35 pg/ml</td>
<td>4.12 pg/ml</td>
<td>1.37 pg/ml</td>
<td>0 pg/ml</td>
</tr>
</tbody>
</table>

Preparation of Biotinylated Detection Antibody
(Preparation, Step 6)

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer (Item E2) 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 Diluent Buffer (Item E2) and used in Procedure, step 4.

Dilution of HRP-Streptavidin Concentrate
(Preparation, Step 7)

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Diluent Buffer (Item E2).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1X Assay Diluent to prepare a 600-fold diluted HRP-Streptavidin solution (don’t store the diluted solution for next day use). Mix well.
Sandwich Assay Procedure

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 and sample into appropriate wells. Cover wells 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 Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash procedure as in step 3.
6. Add 100 µl of prepared Streptavidin solution to each well. Cover wells and 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. Cover wells and 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 absorbance at 450 nm immediately.

Fig 2: Example of the Sandwich ELISA process
**Typical Data**

The minimum detectable dose of Human IL-6 was determined to be 3 pg/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

**Sensitivity**

**Recovery**

Recovery was determined by spiking various levels of Human IL-6 into the sample types listed below. Mean recoveries are as follows:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue lysate</td>
<td>92.78</td>
<td>81-101</td>
</tr>
<tr>
<td>Cell lysate</td>
<td>93.65</td>
<td>82-102</td>
</tr>
</tbody>
</table>
## LINEARITY

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Tissue Lysate</th>
<th>Cell lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2 Average % of Expected Range (%)</td>
<td>92</td>
<td>82-102</td>
</tr>
<tr>
<td>1:4 Average % of Expected Range (%)</td>
<td>93</td>
<td>83-103</td>
</tr>
</tbody>
</table>

**Intra-Assay Reproducibility** CV < 10%

**Inter-Assay Reproducibility** CV < 12%

**Specificity** The antibody pair provided in this kit recognizes human IL-6.

This certificate is system generated and does not require a signature

Product of USA, MSDS available
For research use only. Not for use in diagnostic procedures.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich Publications. Purchaser must determine the suitability of the product(s) for their particular use.

Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip