ELISA Flex kit for quantitative determination of native and recombinant mouse IL-5 in solution, e.g. cell supernatant and serum/plasma samples.

### The kit includes

<table>
<thead>
<tr>
<th>Item</th>
<th>3391-1H-6 for 6 plates</th>
<th>3391-1H-20 for 20 plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture mAb: TRFK5 (1 mg/ml)</td>
<td>150 μl</td>
<td>500 μl</td>
</tr>
<tr>
<td>Detection mAb: TRFK4, biotin (1 mg/ml)</td>
<td>80 μl</td>
<td>250 μl</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
<td>80 μl</td>
<td>250 μl</td>
</tr>
<tr>
<td>Recombinant mouse IL-5 ELISA standard</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>Standard reconstitution buffer A5</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

To ensure total recovery of the stated quantity, vials have been overfilled.

### Shipping and storage

Shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard which should be stored at -20 °C. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.
General and Preparations

Specificity
The kit contains a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant mouse IL-5.

Standard range
1-200 pg/ml

Calibration
No international standard exists for calibration.

Reconstitution of ELISA standard
Reconstitute the ELISA standard to a stock solution of 0.5 µg/ml by adding 0.5 ml of the standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of standard curve
Prepare within 30 minutes of use. Volumes are sufficient for duplicates.
Protocol

Day 1
1. Add 100 µl/well of capture mAb TRFK5 diluted to 0.5 µg/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °C.

Day 2
2. Empty the plate and add 200 µl/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 µl/well).
4. Add 100 µl/well of samples or standards diluted in incubation buffer. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100 µl/well of detection mAb TRFK4-biotin diluted to 1 µg/ml in incubation buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100 µl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. Please note that sodium azide used in buffers will inhibit HRP activity.
9. Wash as above.
10. Add 100 µl/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
11. Add 100 µl/well of 0.2 M H₂SO₄ to stop the reaction.
12. Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.

Mouse IL-5

<table>
<thead>
<tr>
<th>Standard concentration (pg/ml)</th>
<th>OD (450-650 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
</tr>
</tbody>
</table>
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