Human IL-17F ELISA

Cat. No: KB1081
Ver2.0

ELISA Set for Accurate Quantitation from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

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Introduction:

IL-17 family members are glycoproteins secreted as dimers that induce local cytokine production and recruit granulocytes to sites of inflammation. IL-17 is induced by IL-15 and IL-23, mainly in activated CD4+ T cells distinct from Th1 or Th2 cells. IL-17F is the most homologous to IL-17, but is induced only by IL-23 in activated monocytes. IL-17F stimulates production of IL-6, IL-8, GCSF, and regulates cartilage matrix turnover by increasing matrix release and inhibiting new matrix synthesis. IL-17F also inhibits angiogenesis and induces production of IL-2, TGF-β, and monocyte chemoattractant protein1 in endothelial cells.

Intended Use:

The Human IL-17F ELISA is an enzyme-linked immunosorbent assay for accurate and precise quantitative detection of Human IL-17F from samples including serum, plasma, and supernatants from cell cultures. The Human IL-17F ELISA is for research use only. Not for diagnostic or therapeutic procedures.

Materials Provided:

1. Microtiter Coated Plate (96 wells) – 1 no
2. Recombinant Human IL-17F Standard, 1μg/ml, 25μl – 4 vial
3. Human IL-17F Biotin Conjugated Detection Antibody, 50μl – 2 vial
4. Concentrated Streptavidin Horseradish Peroxidase, 50μl - 1 vial
5. Wash Buffer (20X) – 25ml
6. Assay Buffer (5X) – 10ml
7. TMB Substrate – 12ml
8. Stop Solution – 12ml

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50μl to 1000μl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Lin-Lin graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:
1. Store main kit components at 2-8°C.

2. Store recombinant Standard and Detection Antibody at -20°C. Upon thawing, aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements. After reconstitution do not refreeze as the activity of the same will be lost.

3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

- **Cell Culture Supernatant:** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

- **Serum:** Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

- **Plasma:** Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles.

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. **Wash Buffer (1X)**
   - Dilution: To make Wash Buffer (1X), add 5ml of Wash Buffer (5X) to 95ml of DI water. This is the working solution.

2. **Assay Diluent (1X)**
   - Dilution: To make Assay Diluent (1X), add 1ml of Assay Diluent (5X) to 4ml of DI water. This is the working solution.

3. **Detection Antibody**
   - Dilution 1:100 add 50µl of Detection Antibody to 4950µl of Assay Diluent (1X) to make final volume to 10ml.*

4. **Streptavidin-HRP**
   - Dilution 1:200 add 50µl of Streptavidin HRP to 9950µl of Assay Diluent (1X) to make final volume to 10ml.*

5. Upon first use, thaw 1µg tube of recombinant standard and quick-spin, aliquot into polypropylene vials, and store at -20°C. To run the assay, thaw and dilute the recombinant protein by adding 20µl of standard solution in 10ml of Assay Diluent (1X) to prepare the top standard solution (2000pg/ml).

*Note: It is recommended due to low volumes quick-spin down the sample for its settlement at the bottom of the vial provided, before dilution.
Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. Add 100μl/well of Standards and Samples to the plate. Perform six two-fold serial dilutions of the 2000pg/ml top standard, either within the plate or in separate tubes. Thus, the Human IL-17F standard concentrations are 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml and 31.3pg/ml. Assay Diluent (1X) serves as the zero standard (0pg/ml). Seal plate and incubate at 37°C for 1 hour and 30 minutes.

3. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add 100μl of diluted Detection Antibody solution to each well, seal plate and incubate at 37°C for 1 hour and 30 minutes.

5. Wash plate 4 times with Wash Buffer (1X) as in step 3.

6. Add 100μl of diluted Streptavidin-HRP solution to each well, seal plate and incubate at 37°C for 30 minutes.

7. Wash plate 4 times with Wash Buffer (1X) as in step 3. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

8. Add 100μl of freshly mixed TMB Substrate solution and incubate in the dark for 15 – 30 minutes. Monitor the color development at every 5 minutes.

9. Stop reaction by adding 100μl of Stop Solution to each well. Positive wells should turn from blue to yellow.

10. Read absorbance at 450 nm within 30 minutes of stopping reaction.
Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on Lin-Lin graph paper, with cytokine concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown cytokine concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.

![Standard Curve Image]

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.
### Troubleshooting:

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
<th>Investigation/Actions</th>
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</thead>
<tbody>
<tr>
<td><strong>High Absorbances</strong></td>
<td></td>
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</tr>
<tr>
<td>1.</td>
<td>Cross-contamination from other specimens</td>
<td>&gt; Repeat assay taking care when washing and pipetting.</td>
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<tr>
<td>2.</td>
<td>Insufficient or inefficient washing or reading</td>
<td>&gt; Check washer efficiency</td>
</tr>
<tr>
<td>3.</td>
<td>Wavelength of filter not correct.</td>
<td>&gt; Check that the wavelength is 450nm. If a dual wavelength spectrophotometer is available, set the reference filter between 600-650 nm.</td>
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<tr>
<td>4.</td>
<td>High assay background.</td>
<td>&gt; Repeat assay and include a well that contains only sample diluent or sample absorbent (i.e. a blank well).</td>
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<tr>
<td>5.</td>
<td>Contaminated TMB</td>
<td>&gt; Check that TMB is colorless or faint blue.</td>
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<tr>
<td>6.</td>
<td>Incubation time too long or incubation temperature too high.</td>
<td>&gt; Check incubator is at the correct temperature.</td>
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<tr>
<td>7.</td>
<td>Incorrect dilution of serum</td>
<td>&gt; Repeat assay, ensuring correct serum dilution is used.</td>
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<tr>
<td></td>
<td><strong>Low Absorbances</strong></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Incubation time too short or incubation temperature too low.</td>
<td>&gt; Ensure time and temperature of assay incubation are correct.</td>
</tr>
<tr>
<td>2.</td>
<td>Incorrect dilution or pipetting of sera</td>
<td>&gt; Check incubator is set at the correct temperature.</td>
</tr>
<tr>
<td>3.</td>
<td>Incorrect filter wavelength.</td>
<td>&gt; Ensure controls are sufficiently mixed.</td>
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<td>4.</td>
<td>Contaminated Conjugate solution.</td>
<td>&gt; Dispense conjugate directly from the bottle using clean pipette tips; avoid transferring Conjugate to another container if possible.</td>
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<tr>
<td>5.</td>
<td>Kit has expired.</td>
<td>&gt; Do not return unused Conjugate to bottle.</td>
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<tr>
<td>6.</td>
<td>Air blank reading high.</td>
<td>&gt; Ensure all pipettes and probes used to dispense the Conjugates are clean and free from serum, detergent and bleach.</td>
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<tr>
<td>7.</td>
<td>Incorrect storage of kit.</td>
<td>&gt; Investgate causes of high background absorbance.</td>
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<tr>
<td>8.</td>
<td>Kit reagents not equilibrated at room temperature</td>
<td>&gt; Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue/purple.</td>
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<tr>
<td>9.</td>
<td>Incorrect reagents used.</td>
<td>&gt; Allow sufficient time for reagents to equilibrate to room temperature prior to assay.</td>
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<tr>
<td>10.</td>
<td>Over washing of plate (e.g. inclusion of a long soak step).</td>
<td>&gt; Check the reagents used match those listed on the specification sheet.</td>
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<td></td>
<td><strong>Poor Duplicates</strong></td>
<td></td>
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<tr>
<td>1.</td>
<td>Poor mixing of samples.</td>
<td>&gt; Mix reagents gently and equilibrate to Room Temperature.</td>
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<tr>
<td>2.</td>
<td>Poor pipette precision</td>
<td>&gt; Calibration may need to be checked.</td>
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<tr>
<td>3.</td>
<td>Addition of reagents at inconstant timing intervals; reagent addition takes too long, air bubbles when adding reagents.</td>
<td>&gt; Check pipetting technique-change pipette tip for each sample and ensure excess liquid is wiped from the outside of the tip.</td>
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<tr>
<td>4.</td>
<td>Inefficient washing - Wash buffer left in wells, inconsistent washing, inadequate washing.</td>
<td>&gt; Use consistent timing when adding reagents.</td>
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<td>5.</td>
<td>Reader not calibrated or warmed up prior to plate reading.</td>
<td>&gt; Ensure all dilutions are made before commencing addition to plate.</td>
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<tr>
<td>6.</td>
<td>Optical pathway not clean</td>
<td>&gt; Improve pipetting technique and skill.</td>
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<tr>
<td>7.</td>
<td>Spillage of liquid from wells</td>
<td>&gt; Tap out wash buffer after washing.</td>
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<td>8.</td>
<td>Serum samples exhibit microbial growth, haemolysis or lipaemia.</td>
<td>&gt; Check wells are sufficiently and uniformly filled and aspirated when washing.</td>
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<td>9.</td>
<td>Uneven well volumes due to evaporation.</td>
<td>&gt; Gently wipe bottom of plate.</td>
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<td></td>
<td>&gt; Check reader light source and detector are clean.</td>
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<td></td>
<td>&gt; Repeat assay, taking care not to knock the plate or splash liquid.</td>
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<td></td>
<td></td>
<td>&gt; It is not recommended to use serum samples exhibiting microbial growth, haemolysis or lipaemia.</td>
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<td>&gt; Cover plate with a lid or plate sealer (not provided).</td>
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</table>
Human IL-17F ELISA

All wells yellow
1. Contaminated TMB.
2. Contaminated reagents (e.g. Conjugate, Wash buffer).
3. Incorrect dilution of serum.
4. Incorrect storage of kit.
5. Inefficient washing- Wash buffer left in wells, inconsistent washing, inadequate washing.
6. If Conjugate reconstitute is required – Conjugate reconstituted incorrectly.

All wells negative
1. Test not performed correctly – correct reagents not added or not added in the correct sequence.
2. Contaminated Conjugate solution.
3. Over- washing of plate (e.g. inclusion of a long soak step).
4. Incorrect storage of kit.
5. Wash Buffer made up with Stop Solution

> Check TMB is colorless or faint blue.
> Check reagents for turbidity.
> Repeat assay, ensuring correct serum dilution is used.
> Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple.
> Tap out wash buffer after washing.
> Check wells are sufficiently and uniformly filled an aspirated when washing.
> Repeat assay ensuring Conjugate is reconstituted according to assay method.

> Check procedure and check for unused reagents.
> Ensure that Stop Solution was not added before Conjugate or TMB.
> Ensure that serum was diluted in correct Sample diluent; e.g. do not use Sample Absorbent for an IgG ELISA.
> Dispense Conjugate directly from the bottle using a clean pipette tip; avoid transferring Conjugate to another container if possible.
> Do not return unused Conjugate to bottle.
> Ensure all pipettes and probes used to dispense the Conjugate are clean and free from serum, detergent and bleach.
> Repeat assay using recommended wash procedure.
> Ensure kit is stored at 2-8°C, plate is sealed in foil pouch and desiccant sachet is blue / purple.
> Ensure Wash Buffer is made up correctly.

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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