IL-17 ELISA Kit

Catalog Number KAC1591 (96 tests)

Pub. No. MAN0019484 Rev. 1.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the ELISA Technical Guide (Pub. No. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ IL-17 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of IL-17 in serum, plasma, cell culture supernatant, and other biological fluids. The assay recognizes both natural and recombinant IL-17.

Interleukin-17 (IL-17) is a 155 amino acid polypeptide secreted by activated CD4+ T Cells as a mixture of homodimeric glycosylated and non-glycosylated polypeptides. IL-17 may constitute an early initiator of the T Cell-dependent inflammatory reaction and an essential element of the cytokine network that bridges the immune system to hematopoiesis. IL-17 additionally plays a limited pro-inflammatory role in T-cell driven inflammatory pathological processes such as psoriasis or sarcoidosis.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. Store the Wash Solution Concentrate at room temperature. When stored as indicated, all reagents are stable until the expiration date.

Contents	Cat. No. KAC1591 (96 tests)
Solution A, human plasma with preservatives	2 vials
Solution B, buffer with preservatives	22 mL
Controls 1 and 2 in human plasma with preservatives; lyophilized. Refer to vial label for reconstitution volume and range.	2 vials
Calibrator (Standard); in bovine plasma with preservatives. Refer to vial label for exact concentration.	2 vials
IL-17 Antibody-Coated Wells, 96-well strip-well plate	1 plate
Anti-IL-17 Biotin Conjugate, in a buffered solution with proteins and preservatives	6 mL
SAV-HRP Diluent	22 mL
SAV-HRP Concentrate (51X)	0.3 mL
Wash Solution Concentrate (200X)	10 mL
Chromogenic TMB (tetramethylbenzidine) in DMF	25 mL
Stop Solution (1 N HCl)	25 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Horizontal microplate shaker capable of 700 rpm ± 100 rpm
- Magnetic stirrer

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 2 mL of Wash Solution Concentrate (200X) with 398 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Use a magnetic stirrer to mix the solution.

   Note: Use 1X Wash Buffer on the same day it is prepared. Discard unused 1X Wash Buffer at the end of the day.

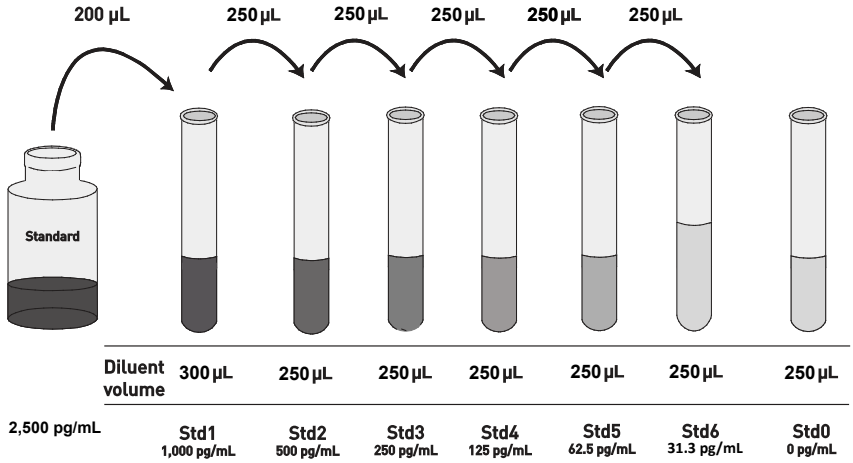
Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Reconstitute standards

Note: Standards are stable for 4 days at 2–8°C. For long-term storage, make aliquots and store at –20°C for up to 2 months. Avoid freeze/thaw cycles.

- Reconstitute the lyophilized standard to 2500 pg/mL with distilled water. Refer to the standard vial label for instructions. Swirl or mix gently to ensure complete reconstitution, then make serial dilutions as described below.



Reconstitute Solution A and Controls

Note: Controls are stable for 4 days at 2–8°C. For long-term storage, make aliquots and store at –20°C for up to 2 months. Avoid freeze/thaw cycles.

- Reconstitute the lyophilized Solution A and Controls to the volume specified on the vial label with distilled water. Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion.

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy can be determined.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

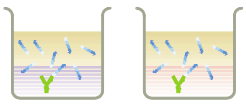
- Dilute the sample with Specimen Diluent as needed.
  - **a.** For serum and plasma samples, dilute with reconstituted Solution A.
  - **b.** For cell culture supernatant and urine samples, dilute with Solution B or the type of culture medium used to grow the cells.
- If samples generate values higher than the highest standard, dilute samples further and repeat the assay.

Perform ELISA (Total assay time: 2.75 hours)

IMPORTANT! Perform a standard curve with each assay.

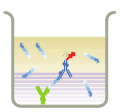
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



- **a.** Pipet 50 µL of Solution B into the appropriate wells for the standards, controls, and serum/plasma samples.
- **b.** Pipet 50 µL of Solution A into the appropriate wells for cell culture supernatant samples.
- **c.** Add 100 µL of standards, controls, or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
- **d.** Pipet 50 µL of Anti-IL-17-Biotin conjugate into all of the wells.
- **e.** Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
- **f.** Thoroughly aspirate the solution and wash wells 3 times by with 1X Wash Buffer.

2 Add HRP Conjugate solution



- **a.** Add 100 µL of diluted Streptavidin-HRP conjugate into all the wells.
- **b.** Incubate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
- **c.** Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3	Add Chromogenic TMB 	**a.** Add 100 µL of Chromogenic TMB to each well. The substrate solution begins to turn blue. **b.** Incubate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm in the dark. **Note:** TMB should not touch aluminum foil or other metals.
4	Add Stop Solution 	Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 1 hour after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data obtained for standards are for illustration only and should never be used in place of a real time standard curve.

Concentration (pg/mL)	Optical Density (450 nm)
1000	2.38
500	1.26
250	0.64
125	0.34
62.5	0.19
31.3	0.11
15.6	0.07
0	0.02

Inter-assay precision

Inter-assay data in process.

Intra-assay precision

Samples of IL-17 were assayed in multiple assays to determine precision within an assay.

Parameters	Sample 1	Sample 2
Standard Deviation (pg/mL)	169 ± 6	456 ± 17
% Coefficient of Variation	3.7	3.7

Expected values

Expected values are in process.

Sensitivity

The minimum detectable dose of IL-17 is 2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

No significant cross-reaction was observed in presence of 300 ng of IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-16, GM-CSF, TNF-α, IFN-γ, TGF-β, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, GRO, IP-10, SCF, MCP-3, NAP-2, and RANTES. This kit is specific for natural and recombinant IL-17.

Recovery

Sample	Added IL-17 (pg/mL)	Recovery IL-17 (pg/mL)	Recovery %
Plasma	882	741	84
	375	323	86
	187	175	94
High Rheumat. Factor Sample	882	702	80
	375	351	94
	187	242	29
Cell Culture Medium	882	883	100
	375	413	110
	187	217	116

High-dose hook effect

A sample spiked with IL-17 up to 0.2 µg/mL gives a response higher than that obtained for the last standard point.

Linearity of dilution

Dilution	Cell Culture 1			Cell Culture 2		
	Measured conc. (pg/mL)	Theor. conc. (pg/mL)	Recovery (%)	Measured conc. (pg/mL)	Theor. conc. (pg/mL)	Recovery (%)
1/1	450	450	—	630	630	—
1/2	232	225	103	326	315	103
1/4	120	113	100	156	158	99
1/8	54	56	96	85	79	108
1/16	25	28	89	36	40	90

Dilution	Activated Plasma		
	Measured conc. (pg/mL)	Theor. conc. (pg/mL)	Recovery (%)
1/2	597	630	95
1/4	256	315	81
1/8	130	158	82
1/16	60	79	76
1/32	33	40	83

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.