Human Eotaxin ELISA Kit

Catalog Number KAC2231 (96 tests)

Pub. No. MAN0005809 **Rev.** 2.0

CAUTION! This kit contains materials with small quantities of sodium azide and Proclin™ 300. 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. In case of contact, rinse affected area with plenty of water. Proclin™ 300 is toxic, corrosive, and a skin irritant. 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™ Human Eotaxin ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human eotaxin in human serum, EDTA plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant human eotaxin.

Human eotaxin is a 8.3 kDa (73 amino acid) non-glycosylated polypeptide. Eotaxin is closely related to MCPs (Monocyte Chemotactic Proteins) and thus is considered to be a member of the β (or CC) chemokine subfamily. Eotaxin exhibits an inter-species homology of 60% between human, mouse, and guinea pig. Human eotaxin shows 50–70% identity at the amino acid and nucleotide level with human MCP-1, MCP-2, and MCP-4; ~30% with RANTES, MIP-1α, and HCC-1; and ~35% amino acid identity with the functionally similar eotaxin-2.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KAC2231 (96 tests)
Hu Eotaxin Standard; lyophilized. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 7.7 mM sodium azide	25 mL
Hu Eotaxin Antibody-Coated Wells, 96-well strip-well plate	1 plate
Hu Eotaxin Biotin Conjugate; contains 7.7 mM sodium azide	11 mL
Streptavidin-Peroxidase (HRP) (100X); contains 0.04% Proclin™ 300, 3.3 mM thymol	0.125 mL
Incubation Buffer; contains 7.7 mM sodium azide	11 mL
Streptavidin-Peroxidase (HRP) Diluent; contains 0.04% Proclin™ 300 and 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

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 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

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.

Pre-dilute samples

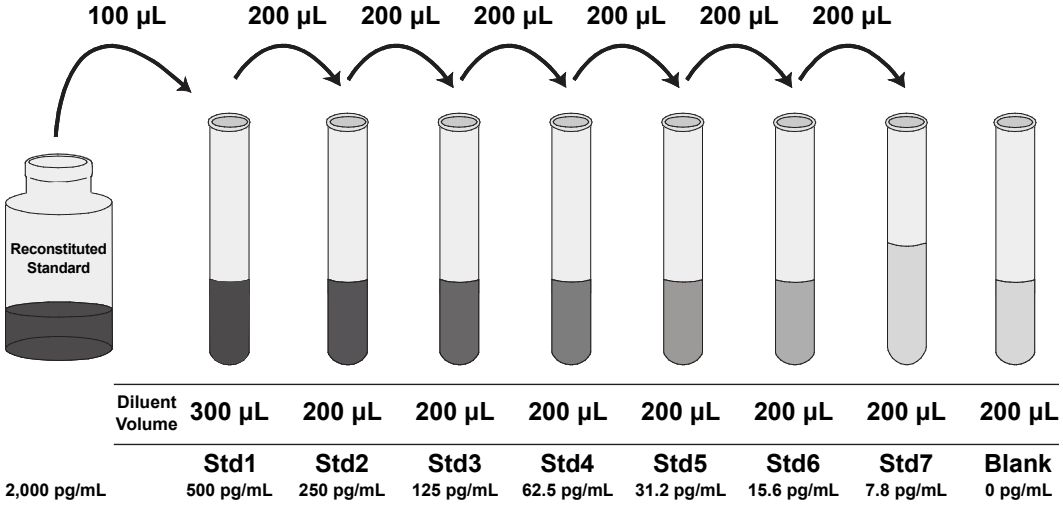
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Perform sample dilutions with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu Eotaxin Standard to 2,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2,000 pg/mL human eotaxin. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 300 µL Standard Diluent Buffer and mix. Label as 500 pg/mL human eotaxin.
3. Add 200 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.2, 15.6, 7.8 and 0 pg/mL human eotaxin.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

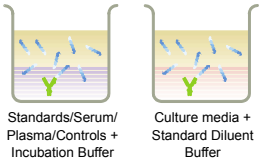
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 3 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. Add 50 µL of **Incubation Buffer** to wells for standards, serum/plasma, and controls.
b. Add 50 µL of **Standard Diluent Buffer** to wells for cell culture media.
c. Add 50 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. Add 100 µL Hu Eotaxin Biotin Conjugate solution into each well except the chromogen blanks.
e. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
f. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP

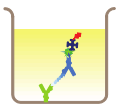
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature 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 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four 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 were obtained for the various standards over the range of 0 to 500 pg/mL human eotaxin.

Standard Human Eotaxin (pg/mL)	Optical Density (450 nm)
500	3.15
250	1.59
125	0.89
62.5	0.46
31.2	0.26
15.6	0.15
7.8	0.09
0	0.04

Inter-assay precision

Samples were assayed 8 times in 5 different assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	85.4	203.4	345.3
Standard Deviation	3.5	10.0	20.1
% Coefficient of Variation	4.0	4.9	5.8

Intra-assay precision

Serum-based and buffer-based samples of known human eotaxin concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	92.0	219.4	375.1
Standard Deviation	3.8	10.9	13.9
% Coefficient of Variation	4.1	4.9	3.7

Expected values

Each laboratory must establish its own normal values. For guidance, the mean of normal sera and plasma samples was measured with the Human Eotaxin ELISA Kit.

Sample	Range (pg/mL)	Average (pg/mL)
Serum (n=16)	71.2–247.8	133
EDTA plasma	42.1–141.4	74.1
Citrate plasma (n=16)	85.4–209.2	138

No eotaxin was detectable from human peripheral blood mononuclear cells cultured for up to 72 hours in RPMI supplemented with 5% FCS, after a variety of stimulation conditions including PMA/ionophore (0.1 µg/mL each) or PHA (5 µg/mL), and LPS (25 µg/mL).

Linearity of dilution

Human serum containing 222 pg/mL of measured human eotaxin was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.999.

Recovery

The recovery of human eotaxin added to normal human serum, normal human plasma, or cell culture medium containing fetal bovine serum (FBS) was measured with the Human Eotaxin ELISA Kit.

Sample	Average % Recovery
Serum	100
Plasma	88
Cell culture medium + 1% FBS	85
Cell culture medium + 10% FBS	90

Sensitivity

The analytical sensitivity of the assay is <2.2 pg/mL human eotaxin. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times.

Specificity

Buffered solutions of a panel of substances at 150 ng/mL were assayed with the Human Eotaxin ELISA Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, G-CSF, M-CSF, GM-CSF, GRO, SCF, IP-10, IFN-α, IFN-γ, LIF, MIP-1α, MIP-1β, MCP-1, MCP-3, MCP-4, OSM, RANTES, TNF-α, EGF.

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 found on Life Technologies' website 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

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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