Human IL-15 ELISA Kit

EZHIL15

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Introduction

IL-15 was discovered in the supernatant from a simian kidney epithelial cell line CV-1/EBNA, as a soluble factor capable of supporting proliferation of the IL-2- dependent cell line, CTLL-2. Interleukin-15 (IL-15) is a regulatory cytokine, and it is produced by dendritic cells, epithelial cells, human stromal cell line (IMTLH), fibroblasts, and monocytes. IL-15 plays an important role in immune response and shares many functions with IL-2. In addition, IL-15 promotes the development of dendritic cells, activates human neutrophils and induces the production of proinflammatory cytokines from macrophages. IL-15 acts as a bridge between innate and adaptive immunity because of its diverse roles in the immune system. IL-15 shares with IL-2 the receptor β-chain and γ-chain. The IL-15 receptor complex also includes a specific α subunit (IL-15Rα), distinct from the IL-2Rα chain.

The Human IL-15 ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with 96 well strip plates that are pre-coated with a capture antibody. The ELISA kits with pre-coated plates are specifically designed for the accurate quantification of analytes from cell culture supernatant, serum, plasma or other body fluids. All kits are analytically validated and provide ready-to-use reagents.

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Materials Provided

Store at 2°C to 8°C		
Component	**Item No.**	**Volume**
Anti-Human IL-15 pre-coated 96-well plate	CS211397	1 plate
Human IL-15 Detection Antibody	CS211398	1 bottle (12 mL)
Human IL-15 Standard	CS211399	1 vial (lyophilized)
Avidin-HRP A	CS210051	1 bottle (12 mL)
Assay Buffer A	CS210062	1 bottle (25 mL)
Wash Buffer (20X)	CS210053	1 bottle (50 mL)
Substrate Solution E	CS210030	1 bottle (12 mL)
Stop Solution	CS210055	1 bottle (12 mL)
Plate Sealers	CS210056	4 sheets

Materials Required But Not Supplied
- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 mL to 1,000 mL
- Deionized water
- Wash bottle or automated microplate washer
- Log-log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

Warnings and Precautions

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and plasma in accordance with NCCLS regulations.
- Stop Solution contains 2N sulfuric acid. *Wear eye, hand, and face protection.*
- Before disposing the plate at end of experiments, rinse the plate with copious amount of tap water.

Storage and Stability

Store at 2°C-8°C; performance guaranteed for 4 months from date of receipt when reagents are stored properly.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine an optimal dilution factor to ensure accurate quantification.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at -70°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x g. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze/thaw cycles.
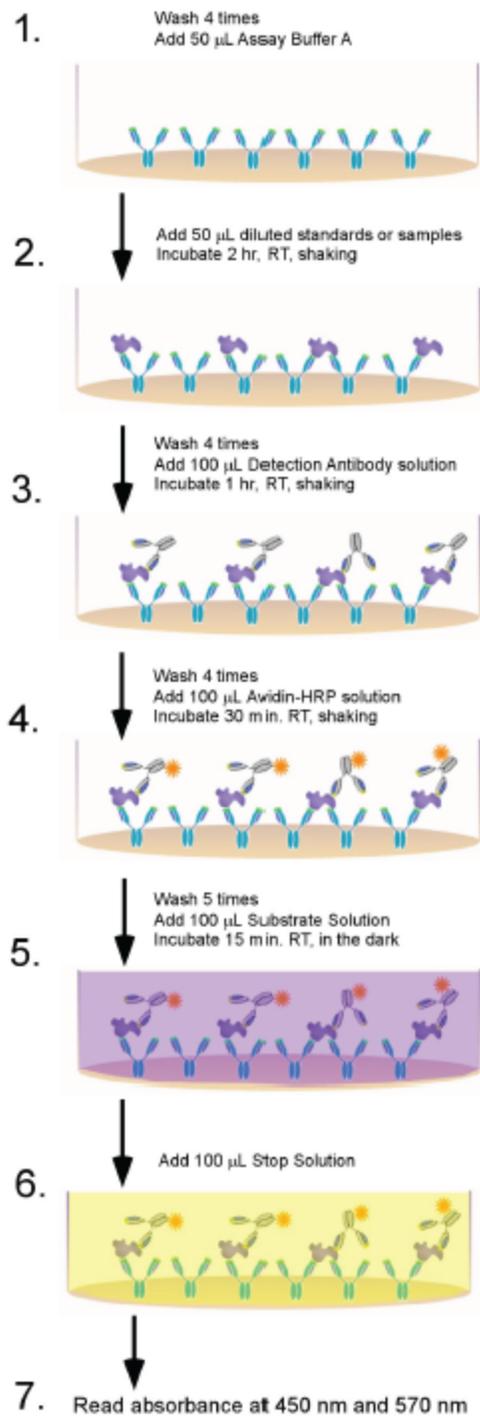
Reagent and Sample Preparation

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water.

2. Reconstitute the lyophilized Human IL-15 Standard by adding the volume of Assay Buffer A described on the vial label to make the 20 ng/mL standard stock solution, mix gently. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, vortex again to mix completely.

3. In general, samples are analyzed without dilutions. If dilutions are necessary, samples should be diluted in Assay Buffer A

Protocol

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

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. Prepare 500 µL of the 500 pg/mL top standard by diluting 12.5 µL of the standard stock solution in 487.5 µL Assay Buffer A. Perform six two-fold serial dilutions of the 500 pg/mL top standard in separate tubes. Thus, the human IL-15 standard concentrations in the tubes are 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL and 7.8 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).

3. Wash plate 4 times with at least 300 µl 1X Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.

4. Add 50 µL of Assay Buffer A to each well that will contain either standards or samples.

5. Add 50 µL/well of standards or samples to the appropriate wells.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours with shaking.

7. Dump the plate contents in a sink then, wash the plate 4 times with 1X Wash Buffer as in step 3.

8. Add 100 µL of Human IL-15 Detection Antibody Solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.

9. Dump the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 3.

10. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes with shaking.

11. Dump the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 3. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

12. Add 100 µL of Substrate Solution E to each well and incubate for 15 minutes in the dark. Wells with higher concentrations of human IL-15 should turn a blue color. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The blue color should change to yellow color.

14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

1. Wash 4 times
   Add 50 μL Assay Buffer A

2. Add 50 μL diluted standards or samples
   Incubate 2 hr, RT, shaking

3. Wash 4 times
   Add 100 μL Detection Antibody solution
   Incubate 1 hr, RT, shaking

4. Wash 4 times
   Add 100 μL Awidin-HRP solution
   Incubate 30 min. RT, shaking

5. Wash 5 times
   Add 100 μL Substrate Solution
   Incubate 15 min. RT, in the dark

6. Add 100 μL Stop Solution

7. Read absorbance at 450 nm and 570 nm

Data Analysis

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration 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 the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:
This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.
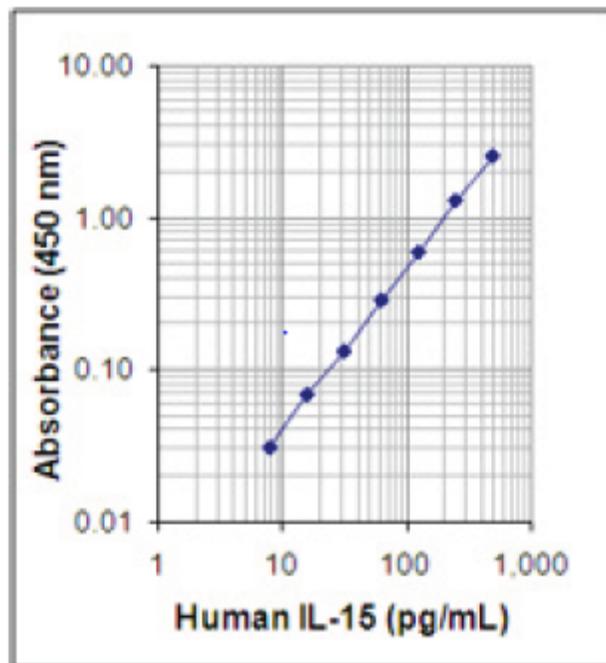
Product Performance

Specificity: No cross reactivity was observed when this kit was used to analyze 14 human recombinant cytokines/chemokines at up to 50 ng/mL.

Sensitivity: The minimum detectable concentration of IL-15 is 2 pg/mL.

Recovery: Recombinant IL-15 (250, 62.5 and 15.6 pg/mL) was spiked into 6 human serum samples, and then analyzed by human IL-15 LEGEND MAX™ Kit. On average, 104 % of the cytokine was recovered from serum samples

Linearity: Three human serum samples with high concentrations of IL-15 were diluted 1:2, 1:4, 1:8 with Assay Buffer A to produce samples with values within the dynamic range and then assayed. On average, 103 % of the expected cytokine was detected from serum samples.

Intra-Assay Statistics: Sixteen replicates each of two samples containing different IL-15 concentrations were tested in one plate.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	291.2	49.5
Standard Deviation	8.3	4.2
% CV	2.9	8.6

Inter-Assay Statistics: Two samples containing different concentrations of IL-15 were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg.mL)	242.6	51.5
Standard Deviation	13.8	5.3
% CV	5.7	10.3

Biological Samples: Human PBMC (1×10^6 cells/mL) were stimulated with 100 ng/mL LPS at 37°C for 2 days. Cell culture supernatants were collected and assayed for the levels of natural human IL-15. IL-15 concentration is 51.2 pg/ mL in LPS-stimulated samples and 3.9 pg/mL in unstimulated samples.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN$_3$)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70℃. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Trouble Shooting Guide:

Problem	Probable Cause	Solutions
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Catalog No. EZHIL15