Human IL-7 ELISA Kit

For the quantitative determination of human interleukin-7 (IL-7) concentrations in serum, plasma, cell culture supernatant

Catalogue Number: EL10041

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (05) IL-7

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INTENDED USE

This Human Interleukin-7 ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin-7 (IL-7) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not to be used in diagnostic or therapeutic procedures.

INTRODUCTION

Human IL-7 is 17.4 kDa protein containing six exons. The gene has a length of approximately 33 kb and maps to chromosome 8q12-q13. Expressed mouse IL-7 is a 25 kDa single-chain glycoprotein. Human (152 amino acids, 17.4 kDa) and murine IL-7 (129 amino acids) display a 60 percent sequence homology at the protein level, and both proteins contain three disulfide bonds that are essential for the biological activity of the molecule. The human IL-7 receptor is an integral, highly glycosylated membrane protein of 76 kDa that is expressed on activated T-cells, which has been designated as CD127.

IL-7 is produced predominantly by epithelial cells, especially keratinocytes and thymic epithelial cells, and is a nonredundant cytokine in thymic T-cell development. IL-7 enhances survival and proliferation of T cell precursors in humans and mice and B cell precursors in mice. IL-7 also affects survival and proliferation of mature T cells, but not mature B cells, and is known to be a potent enhancer of T cell function and IFN-γ production. IL-7 system deficiency in humans may cause severe combined immunodeficiency (SCID) syndrome.

IL-7 is a critical cytokine for normal T and B lymphopoiesis and is a mobilizer of pluripotent stem cells and myeloid progenitors. IL-7 enhances T cell function and induces cytokine expression in monocytes. Preclinical studies indicate that IL-7 is capable of accelerating murine lymphocyte regeneration following chemotherapy and bone marrow transplantation, inducing anti-tumor effects in mice, and expanding anti-HIV-specific human T cells. Because of the potent capability of IL-7 in T-lymphopoiesis augmentation, IL-7 may play a role in the immune reconstitution of T-cells during HIV infection, especially in the context of effective antiretroviral treatments. IL-7 is elevated in untreated patients with advanced HIV disease and in those who fail highly active antiretroviral therapy (HAART), but is undetectable in HIV-infected patients who respond to HAART. Immune reconstitution is a critical issue in the treatment of HIV infection, and immune replacement therapy that relies on a variety of regulatory cytokines has been proposed to reconstitute the immune system function in HIV-infected patients. Thus, it is essential that continued preclinical and clinical research is performed to evaluate IL-7 as a potential therapy for leukopenia, bone marrow/stem cell transplantation, cancer, and HIV/AIDS.

This IL-7 ELISA is a ready-to-use 3.5-hour solid phase immunoassay readily capable of measuring IL-7 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 2000 pg/mL. This assay has shown no cross-reactivity with other cytokines tested, and is expected to be used effectively for further investigations into the relationship between IL-7 and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-7 enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to IL-7. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-7 and incubated. If present, IL-7 will bind and become immobilized by the antibody pre-coated on the wells and then become "sandwiched" by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound IL-7 and other components of the sample. In order to quantitatively determine the amount of IL-7 present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits, each having a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain IL-7, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of IL-7 in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-7 concentration (pg/mL). The concentration of IL-7 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- The Human IL-7 ELISA kit is not for use in clinical diagnostic procedures, and is for laboratory use only.
- Although all manufacturing precautions have been exercised to ensure that this product will be suitable for use with all validated sample types as designated in the product insert, the possibility of interference cannot be excluded due to the variety of proteins that may exist within the sample.

- The Calibrator Diluent selected for the standard curve should be consistent with the assay samples. If the values generated by the samples are greater than the uppermost standard, the samples dilution should be adjusted with the appropriate Calibrator Diluent and the assay should be repeated.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **IL-7 MICROTITER PLATE** (Part EL41-1) ***96 wells***
   Pre-coated with anti-human IL-7monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL41-2) ***6 mL***
   Anti-human IL-7 polyclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL41-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-7 STANDARD** (Part EL41-4) ***2 vials***
   Recombinant human IL-7 (4000pg/Vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL41-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing*.

6. **CALIBRATOR DILUENT II** (Part EL41-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL41-7) ***10 mL***
   Buffered solution with H_2O_2.

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.

12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, or EDTA as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulates.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Samples should be measured immediately, or be aliquoted and stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

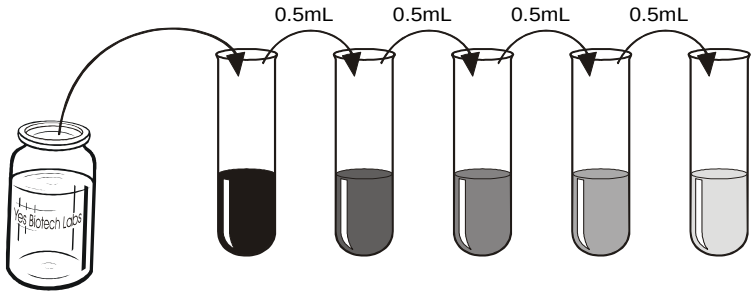
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-7 Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-7 Standard with 2.0 mL of Calibrator Diluent I (for serum / plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 2000 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-7 standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (0 to 2000 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted IL-7 stock solution will serve as the high standard (2000 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



IL-7 Standard	1,000pg/mL	500pg/mL	250pg/mL	125pg/mL	62.5pg/mL
2,000pg/mL					

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-7 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0 pg/mL**	(S1)	**2A, 2B**	Standard 5 - **500 pg/mL**	(S6)
1C, 1D	Standard 2 - **62.5 pg/mL**	(S2)	**2C, 2D**	Standard 6 - **1000 pg/mL**	(S7)
1E, 1F	Standard 3 - **125 pg/mL**	(S3)	**2E, 2F**	Standard 7 - **2000 pg/mL**	(S7)
1G, 1H	Standard 4 - **250 pg/mL**	(S4)	**3A-12H**	**IL-7** samples	

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100 µL of Standard or Sample to the appropriate well of the antibody pre-coated microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Without discarding the standards and samples, add 50 µL Anti-IL7 Biotin Conjugate to each well. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no

moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Add 100 µl of Avidin Conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100 µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100 µL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-7 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-7 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

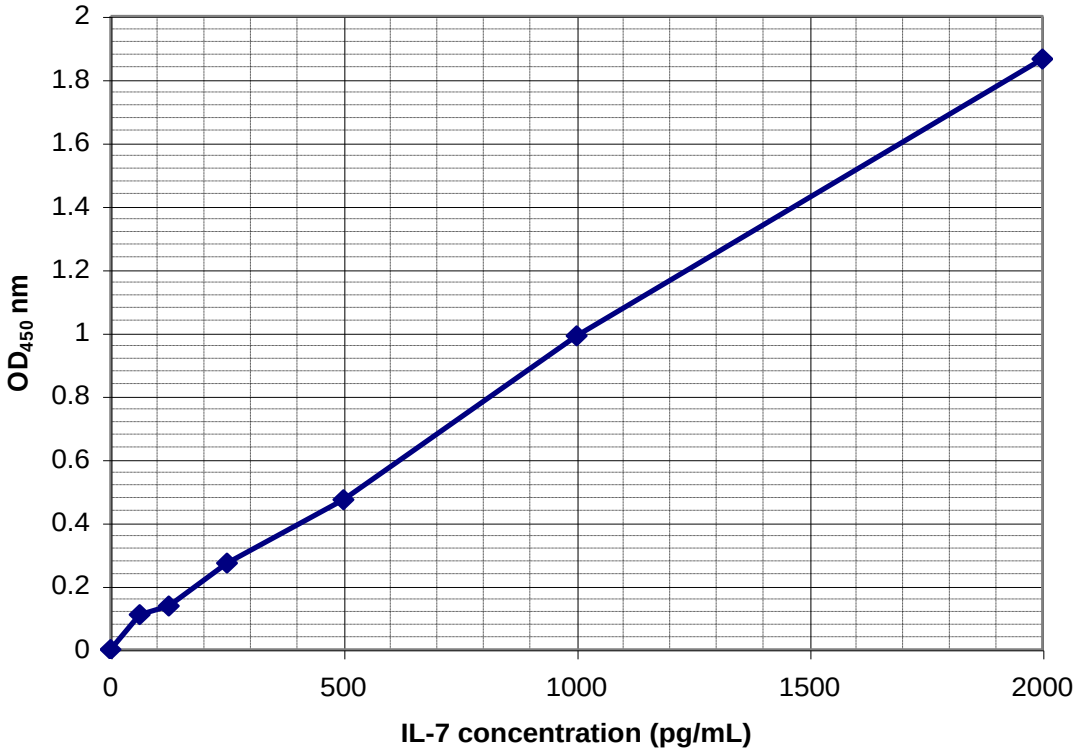
2. To determine the amount of IL-7 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-7 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

Results of a typical standard run of an IL-7 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of ***illustration only***, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE

Standard (pg/mL)	**O.D. (450 nm)**	**Mean**	**Zero Standard Subtracted** (Std.) - (S1)
0	0.081, 0.085	0.083	0
62.5	0.191, 0.196	0.193	0.110
125	0.221, 0.218	0.220	0.137
250	0.280, 0.266	0.273	0.190
500	0.469, 0.477	0.473	0.390
1000	1.069, 1.078	1.073	0.990
2000	1.955, 1.942	1.948	1.865



PERFORMANCE CHARACTERISTICS

1. INTRA - ASSAY PRECISION
   To determine within-run precision, three different samples of known concentration were assayed by using 16 replicates in 1 assay.

	Calibrator Diluent I assay			**Calibrator Diluent II assay**		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
N	16	16	16	16	16	16
Mean(pg/mL)	125	500	1000	125	500	1000
Standard Deviation (pg/mL)	7.6	22	40	7.8	24	37
Coefficient of Variation (%)	6.1	4.4	4.0	6.2	4.8	3.7

2. INTER - ASSAY PRECISION
   To determine between-run precision, three different samples of known concentration were assayed by using replicates on 16 different assays.

	Calibrator Diluent I assay			**Calibrator Diluent II assay**		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
N	16	16	16	16	16	16
Mean(pg/mL)	125	500	1000	125	500	1000
Standard Deviation (pg/mL)	10	26	55	8.9	32	54
Coefficient of Variation (%)	8.0	5.2	5.5	7.1	6.4	5.4

3. SENSITIVITY
   The minimum detectable quantities of human IL-7 are 13.6 pg/mL and 45 pg/mL, when use a standard curve generated with Calibrator Diluent I and Calibrator Diluent II, respectively. The two standard deviations above the mean optical density of the 16 replicates of the zero standard were defined as the minimum detectable quantities.

4. SPECIFICITY
   This sandwich ELISA can detect both natural and recombinant human IL-7. This kit exhibits no significant cross-reactivity with human IL-1, IL-3, IL-4, IL-6, IL-8, G-CSF, GM-CSF, TGF-β1, TGF-β2, TNF-α, TNF-β.

5. CALIBRATION
   This immunoassay is calibrated against natural human IL-7. (NIBSC/WHO First International Standard 90/530).

6. RECOVERY
   The recovery of IL-7 spiked to levels throughout the range of the assay followed by activation in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum	95	91-105

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