Human IL-10 ELISA Kit

For the quantitative determination of human interleukin 10 (IL-10) concentrations in serum, plasma, cell culture supernatant, and other biological fluids

Catalogue Number: EL10027

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03) IL-10

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INTENDED USE

This Human Interleukin 10 ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin 10 (IL-10) 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

The mature human IL-10 is a protein with 160 amino acids and the functional IL-10 is a homodimer. IL-10 is expressed by a variety of cells, including mouse T cells (TH1, TH2, and Tr1 subsets), B cells derived from peripheral blood, tonsils or spleen, Epstein–Barr virus (EBV)-transformed B cell lines, Burkitt's lymphoma, AIDS lymphomas, monocytes, placental trophoblasts, bronchial epithelial cells, and certain tumor cells including melanomas and carcinomas of various origin.

IL-10 plays an important role in inflammatory and immune responses. The biological activities of IL-10 include both immunosuppressive and immuno-stimulatory effects. For example, IL-10 can suppress the production of pro-inflammatory cytokines by monocytes and neutrophils, and down regulates the expression of activating and co-stimulatory molecules on monocytes and dendritic cells. IL-10 also can improve the growth of B cells and mast cells, and inhibit or enhance the activities of T cells depending on their activation conditions. The different polymorphisms in the IL-10 gene promoter have been demonstrated the association with both SIDS (sudden infant death syndrome) and sudden unexpected death due to infection.

Based on the functions of IL-10, it has been suggested that an IL-10 antagonist can be applied to boost anti-viral immunity against viruses such as EBV and that the IL-10 molecule itself may be used as an anti-inflammatory reagent.

This IL-10 ELISA is a ready-to-use 3.5-hour solid phase immunoassay capable of measuring IL-10 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1600 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-10 and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This IL-10 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-10. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated monoclonal antibody preparation specific for IL-10 and incubated. IL-10, if present, will bind and

become immobilized by the antibody pre-coated on the wells and then will become “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound IL-10 and other components of the sample. In order to quantitatively determine the amount of IL-10 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-10, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of IL-10 within 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 standard provided 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-10 concentration (pg/mL). The concentration of IL-10 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- This Human IL-10 ELISA kit is for laboratory research use only, and is not intended for use in clinical diagnostic procedures.
- 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-10 MICROTITER PLATE** (Part EL27-1) ***96 wells***
   Pre-coated with anti-human IL-10 monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL27-2) ***6 mL***
   Anti-human IL-10 monoclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL27-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **IL-10 STANDARD** (Part EL27-4) ***2 vials***
   Recombinant human IL-10 (3200 pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL27-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II (5x)** (EL27-6) ***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 EL27-7) ***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 Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: 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 the 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 or 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

1. 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, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulates.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, and cell culture supernatant samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

2. DILUTION PROCEDURE

Cell culture supernatant samples may require a dilution of 1:10 with Calibrator Diluent II (1x). The suggested dilution procedure is as follows:
50 µL cell culture supernatant sample + 450 µL of Calibrator Diluent II (1x).

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. **Calibrator Diluent II (1x) :** Add 1 volume of Calibrator Diluent II (5x) to 4 volumes of distilled or deionized water. Mix well before use.

2. **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.

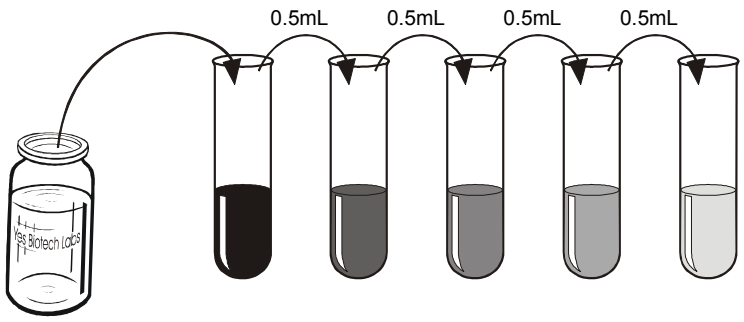
3. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
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

4. **IL-10 Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatent testing. Reconstitute IL-10 Standard with 2.0 mL of Calibrator Diluent I (for serum / plasma testing) or Calibrator Diluent II (1x) (for cell culture supernatant testing). This reconstitution produces a stock solution of 1600 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-10 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 (50 to 600pg/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-10 stock solution will serve as the high standard **(**1600 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



IL-10 Standard 1,600 pg/ml	800 pg/ml	400 pg/ml	200pg/ml	100 pg/ml	50 pg/ml

ASSAY PROCEDURE

Prepare Wash Buffer and IL-10 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	**400 pg/mL**	(S5)
1C, 1D	Standard 2	**50 pg/mL**	(S2)	**2C, 2D**	Standard 6	**800 pg/mL**	(S6)
1E, 1F	Standard 3	**100 pg/mL**	(S3)	**2E, 2F**	Standard 7	**1600 pg/mL**	(S7)
1G, 1H	Standard4	**200 pg/mL**	(S4)	**2G,2H**	**IL-10** 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

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

2. Without discarding the standards and samples, add 50μL IL-10 Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.

3. 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. Dispense 100µl of Avidin Conjugate to each well Mix 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 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100µL 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-10 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-10 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-10 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-10 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.

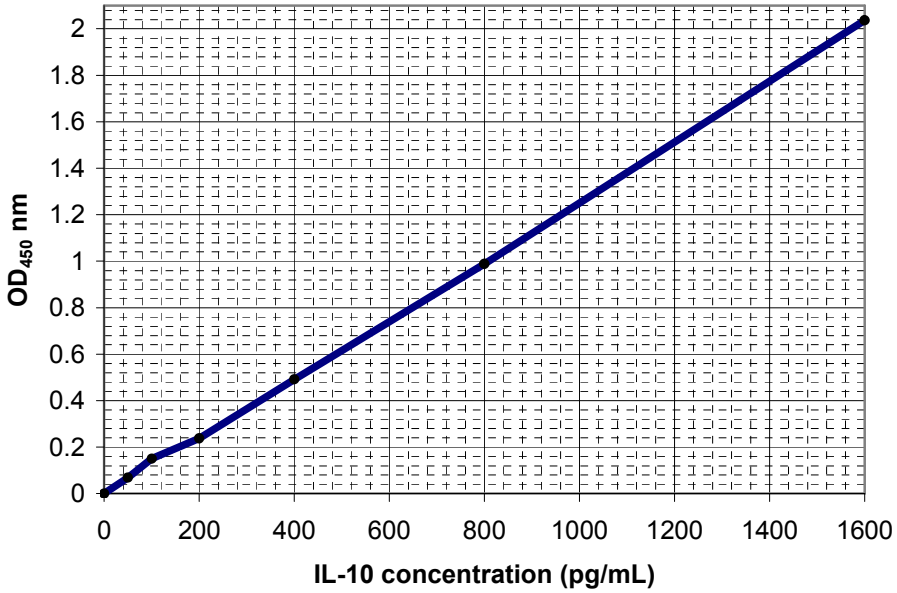
TYPICAL DATA

Results of a typical standard run of an IL-10 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 ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

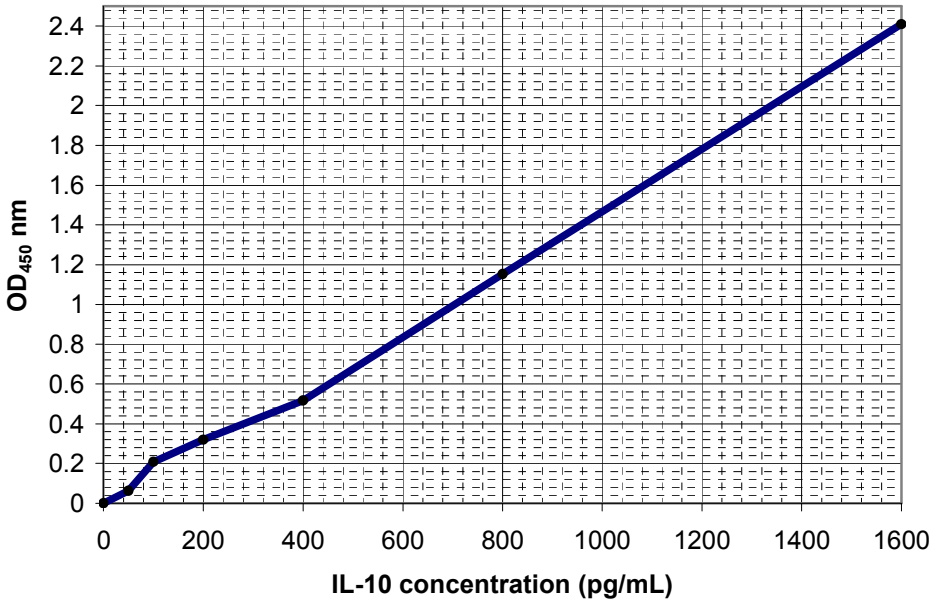
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.110, 0.112	0.111	0
50	0.179, 0.181	0.180	0.069
100	0.265, 0.266	0.261	0.150
200	0.351, 0.341	0.349	0.238
400	0.610, 0.595	0.603	0.492
800	1.098, 1.100	1.099	0.988
1600	2.138, 2.159	2.148	2.037



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.095, 0.092	0.093	0
50	0.156, 0.154	0.155	0.062
100	0.302, 0.300	0.301	0.208
200	0.421, 0.405	0.413	0.320
400	0.611, 0.609	0.610	0.517
800	1.252, 1.240	1.246	1.153
1600	2.512, 2.492	2.502	2.409



PERFORMANCE CHARACTERISTICS

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

	Calibrator Diluent I Assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	32	201	956
Standard Deviation	2.4	8.3	11.7
Coefficient of Variation (%)	*7.5*	*4.1*	*1.2*

	Calibrator Diluent II Assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	36	211	938
Standard Deviation	2.1	8.1	12.5
Coefficient of Variation (%)	*5.8*	*3.8*	*1.3*

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by replicates on 25 different assays.

	Calibrator Diluent I Assay		
Sample	**1**	**2**	**3**
n	25	25	25
Mean (pg/mL)	37	212	908
Standard Deviation	1.8	8.6	12.5
Coefficient of Variation (%)	*4.9*	*4.1*	*1.4*

	Calibrator Diluent II Assay		
Sample	**1**	**2**	**3**
n	25	25	25
Mean (pg/mL)	35	208	917
Standard Deviation	2.5	8.4	12.8
Coefficient of Variation (%)	*7.1*	*4.0*	*1.4*

3. **RECOVERY**
   By employing five random samples, the recovery of IL-10 was evaluated in 7 different amounts of IL-10 throughout the range of the assay. All samples were mixed and assayed in duplicate.

Sample Type	***Average Recovery (%)***	***Range (%)***
Cell Culture Media	98	84-110
Serum	100	92-105
EDTA plasma	90	86-92
Heparin plasma	92	89-94
Citrate plasma	90	81-92

4. **SENSITIVITY**
   The minimum detectable quantities of human IL-10 as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 3.5 pg/mL and 3.7pg/mL respectively. The two standard deviations above the mean optical density of the 20 replicates of the zero standard were defined as the minimum detectable quantities.

5. **SPECIFICITY**
   This sandwich ELISA can detect both natural and recombinant human IL-10. This kit exhibits no significant cross-reactivity with human IL-1α, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNF-α, TNF-β, IFN-γ. There is 2% reaction with recombinant mouse IL-10.

6. **CALIBRATION**
   This immunoassay is calibrated against natural human IL-10. (NIBSC / WHO First International Standard 92/516).

7. EXPECTED NORMAL VALUES
Biological samples from apparently healthy, normal individuals were collected and the average IL-10 concentration was measured. Serum samples (n=15) average value:

3.57±3.70pg/mL, range: 0-14.10pg/mL whereas plasma samples (n=16) average value: 4.51± 4.6 pg/mL, range: 1.25-15.66pg/mLl. Both are less than 20 pg/mL.

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