Revised 29 Dec. 2009 rm (Vers. 3)

Please use only the valid version of the package insert provided with the kit.

Intended Use

The Interleukin-1α (human) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human interleukin-1α (IL-1α).
The human Interleukin-1α ELISA is for in vitro determinations. Not for use in therapeutic procedures.

Summary

The interleukin-1 (IL-1) species represent an important family of biologically active mono nuclear cell-derived proteins which are involved in inflammatory reactions and in immune responses (6, 25). Two distinct human IL-1 species, IL-1α and IL-1β, have been identified (2, 16). They share similarities such as the same molecular weight, similar biological effects and the same receptors on target cells (12, 33). IL-1 proteins are produced by macrophages, monocytes and various other cell types such as adult T cell leukemias (34), fibroblasts, epithelial or endothelial cells (13), neutrophils and astrocytes (8). Their biological properties include pyrogenicity, bone resorption, presentation of antigen to T cells and stimulation of B and T lymphocyte proliferation (20).
IL-1α is an extracellular peptide of 17 kDa, its activity has been demonstrated in various biological fluids (25) including serum, synovial fluid, gingival fluid, amniotic fluid, sputum, cerebrosinal fluid, urine, and bronchoalveolar lavage (BAL) fluid.
Elevated serum or blood levels of IL-1α have been found in patients with total hip replacement/ arthroplasties (14, 30), in patients with recently diagnosted IDDM (9), in case of several carcinomas such as head and neck cancer (15), pancreatic cancer (3) and thyroid cancer (35), in experimental acute pyelonephritis (27), in acute viral hepatitis (31) and in septic shock (1). Both elevations in serum levels and joint fluids (synovial fluids) are detected in rheumatoid arthritis (5, 7, 10, 22, 23, 24). IL-1α elevation is a marker for dental diseases such as pulpal inflammation (21) and infections of the root canals (18).
Pulmonary disorders are accompanied by plasma and BAL elevations of IL-1α, e.g. cystic fibrosis (29, 32), systemic sclerosis (4). Increased plasma and CSF levels are found in patients with schizophrenia (19). Blood levels of newborn with systemic infection during the neonatal period are significantly higher than in controls (26). High concentration of IL-1α in the cervical mucus of pregnant women are found to be involved in defense mechanism against ascending infections (28).
Significantly elevated concentrations in gingival crevicular fluid in subjects with peridantitis are detected (17). Urinary levels of IL-1α correspond to disease and therapy response in bladder cancer (11).

Principles of the Test

An anti-human IL-1α coating antibody is adsorbed onto microwells.
Human IL-1α present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-1α antibody is added and binds to human IL-1α captured by the first antibody.
Following incubation unbound biotin-conjugated anti-human IL-1α antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-1α antibody.
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Revised 29 Dec. 2009 rm (Vers. 3)

A coloured product is formed in proportion to the amount of human IL-1α present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-1α standard dilutions and human IL-1α sample concentration determined.

Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with polyclonal antibody to human IL-1α

1 vial (100 µL) **Biotin-Conjugate** anti-human IL-1α polyclonal antibody

1 vial (150 µL) **Streptavidin-HRP**

2 vials human IL-1α **Standard** lyophilized, 200 pg/mL upon reconstitution

1 vial (12 mL) **Sample Diluent**
Please note: *In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Sample Diluent vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.*

1 vial (5 mL) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)

1 bottle (50 mL) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)

1 vial (15 mL) **Substrate Solution** (tetramethyl-benzidine)

1 vial (15 mL) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 mL) **Blue-Dye**

1 vial (0.4 mL) **Green-Dye**

1 vial (0.4 mL) **Red-Dye**

4 **Adhesive Films**

Storage Instructions – ELISA Kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Specimen Collection and Storage Instructions

Cell culture supernatant, serum, plasma (EDTA, citrate, heparin) and urine were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.
Pay attention to a possible **"Hook Effect"** due to high sample concentrations (see chapter 0).
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IL-1α. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (for sample stability refer to 0).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials Required But Not Provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for in vitro determinations and are not for use in therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

– Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of Reagents

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

Wash Buffer

Pour entire contents (50 mL) of the **Wash Buffer Concentrate** (20x) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.
Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay Buffer (1x)

Pour the entire contents (5 mL) of the **Assay Buffer Concentrate** (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Human IL-1α Standard

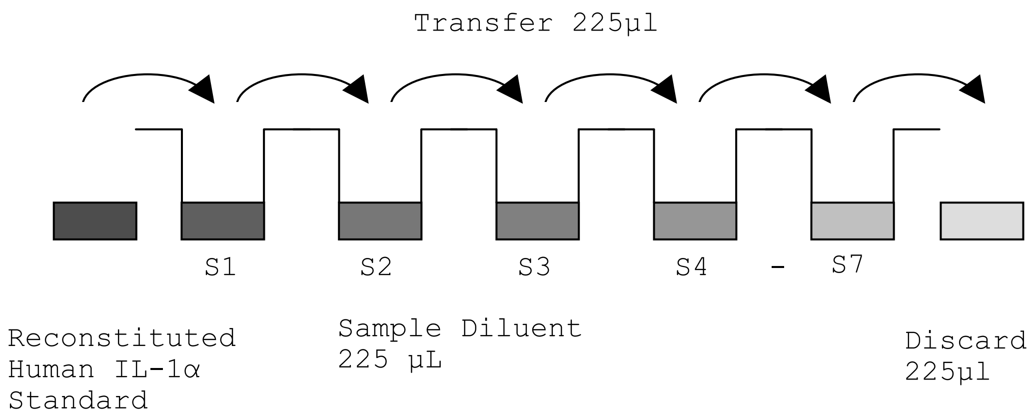
Reconstitute **human IL-1α Standard** by addition of distilled water.
Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200 pg/mL).
Allow the standard to reconstitute for 10 – 30 minutes. Mix well prior to making dilutions.
After usage remaining standard cannot be stored and has to be discarded.

Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 0).

External Standard Dilution

Label 7 tubes, one for each standard point.
S1, S2, S3, S4, S5, S6, S7
Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 225 µL of Sample Diluent into each tube.
Pipette 225 µL of reconstituted standard (concentration = 200 pg/mL) into the first tube, labelled S1, and mix (concentration of standard 1 = 100.0 pg/mL).
Pipette 225 µL of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 1).
Sample Diluent serves as blank.

Figure 1



Addition of Colour-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, a tool is offered that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before standard and sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 mL Sample Diluent	20 µL ***Blue-Dye***
12 mL Sample Diluent	48 µL ***Blue-Dye***
50 mL Sample Diluent	200 µL ***Blue-Dye***

2. Biotin-Conjugate: Before dilution of the concentrated Biotin-Conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet: Preparation of Biotin-Conjugate.

3 mL Assay Buffer (1x)	30 µL ***Green-Dye***
6 mL Assay Buffer (1x)	60 µL ***Green-Dye***
12 mL Assay Buffer (1x)	120 µL ***Green-Dye***

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet: Preparation of Streptavidin-HRP.

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6 mL Assay Buffer (1x)	24 µL ***Red-Dye***
12 mL Assay Buffer (1x)	48 µL ***Red-Dye***

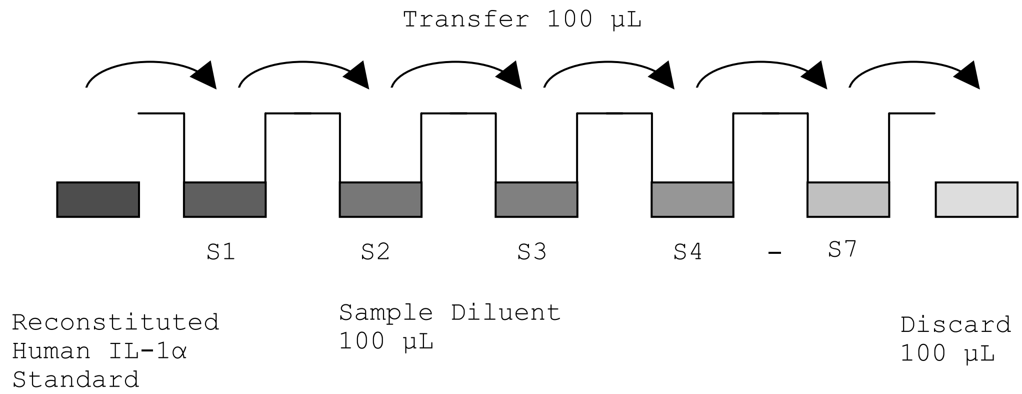
Test Protocol

a. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Wash the microwell strips twice with approximately 400 µL **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes - see 0): Add 100 µL of Sample Diluent in duplicate to all **standard wells**. Pipette 100 µL of prepared **standard** (see Preparation of Standard 0, concentration = 200 pg/mL) in duplicate into well A1 and A2 (see Table 1).
Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 2). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IL-1α standard dilutions ranging from 100.0 to 1.6 pg/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 2



In case of an **external standard dilution** (see 0), pipette 100 µL of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

Table 1:
Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (100.0 pg/mL)	Standard 1 (100.0 pg/mL)	Sample 1	Sample 1
B	Standard 2 (50.0 pg/mL)	Standard 2 (50.0 pg/mL)	Sample 2	Sample 2
C	Standard 3 (25.0 pg/mL)	Standard 3 (25.0 pg/mL)	Sample 3	Sample 3
D	Standard 4 (12.5 pg/mL)	Standard 4 (12.5 pg/mL)	Sample 4	Sample 4
E	Standard 5 (6.3 pg/mL)	Standard 5 (6.3 pg/mL)	Sample 5	Sample 5
F	Standard 6 (3.1 pg/mL)	Standard 6 (3.1 pg/mL)	Sample 6	Sample 6
G	Standard 7 (1.6 pg/mL)	Standard 7 (1.6 pg/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 100 µL of **Sample Diluent** in duplicate to the **blank wells**.

e. Add 50 µL of **Sample Diluent** to the **sample wells**.

f. Add 50 µL of each **sample** in duplicate to the **sample wells**.

g. Prepare **Biotin-Conjugate** (see Preparation of Biotin-Conjugate 0).

h. Add 50 µL of **Biotin-Conjugate** to all wells.

i. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, if available on a microplate shaker set at 200 rpm.

j. Prepare **Streptavidin-HRP** (refer to Preparation of Streptavidin-HRP 0).

k. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.

l. Add 100 µL of diluted **Streptavidin-HRP** to all wells, including the blank wells.

m. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 1 hour, if available on a microplate shaker set at 200 rpm.

n. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b. of the test protocol. Proceed immediately to the next step.

o. Pipette 100 µL of **TMB Substrate Solution** to all wells.

p. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 min. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable.
Determination of the ideal time period for colour development has to be done individually for each assay.
It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.6 – 0.65.

q. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 C - 8°C in the dark.

r. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of Results

Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.
Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-1α concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
To determine the concentration of circulating human IL-1α for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL-1α concentration.
If instructions in this protocol have been followed samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human IL-1α levels (Hook Effect). Such samples require further external predilution according to expected human IL-1α values with Sample Diluent in order to precisely quantitate the actual human IL-1α level.
It is suggested that each testing facility establishes a control sample of known human IL-1α concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3:
Representative standard curve for human IL-1α ELISA. Human IL-1α was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

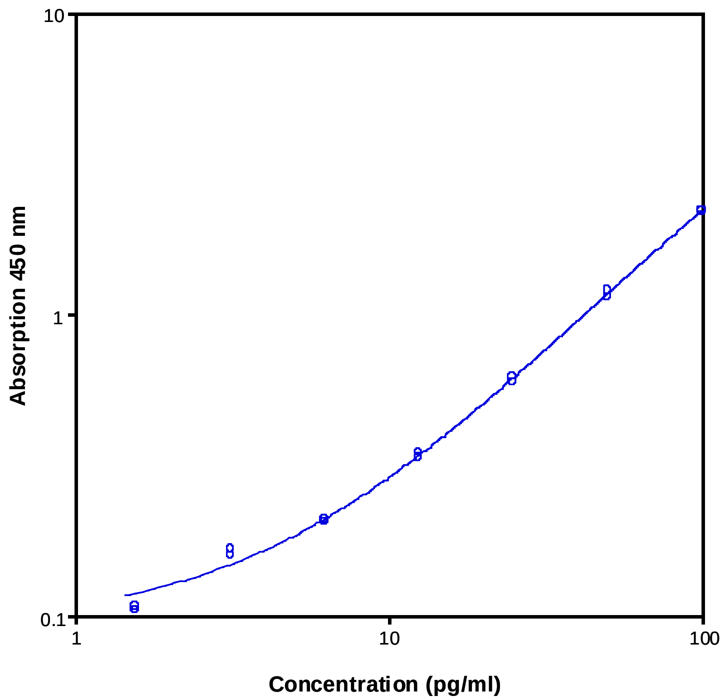


Table 2: Typical data using the human IL-1α ELISA
Measuring wavelength: 450 nm, Reference wavelength: 620 nm

Standard	Human IL-1α Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	100.0	2.206	2.203	0.2
	100.0	2.200		
2	50.0	1.137	1.170	3.9
	50.0	1.202		
3	25.0	0.619	0.608	2.6
	25.0	0.597		
4	12.5	0.348	0.341	2.9
	12.5	0.334		
5	6.3	0.205	0.207	1.0
	6.3	0.208		
6	3.1	0.167	0.163	3.5
	3.1	0.159		
7	1.6	0.108	0.106	2.7
	1.6	0.104		
Blank	0	0.069	0.070	0.7
	0	0.070		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

Limitations

Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance Characteristics

Sensitivity

The limit of detection of human IL-1α defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 1.1 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-1α.
2 standard curves were run on each plate. The calculated overall intra-assay coefficient of variation was < 5.4%.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human IL-1α.
2 standard curves were run on each plate. The calculated overall inter-assay coefficient of variation was < 10%.

Spike Recovery

The spike recovery was evaluated by spiking 4 levels of human IL-1α into different samples. Recoveries were determined in 3 independent experiments with 4 replicates each.
The amount of endogenous human IL-1α in unspiked serum was subtracted from the spike values.
The overall mean recovery was 92%.

Dilution Parallelism

Serum samples with different levels of human IL-1α were analyzed at serial 2 fold dilutions with 4 replicates each.
The overall mean recovery was 99%.

Sample Stability

Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human IL-1α levels determined. There was no significant loss of human IL-1α immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human IL-1α level determined after 24 h. There was no significant loss of human IL-1α immunoreactivity detected during storage at -20°C and 2-8°C.
A significant loss of human IL-1α immunoreactivity (50%) was detected during storage at RT and 37°C after 24 h.

Specificity

The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human IL-1α positive serum.
There was no cross reactivity or interference detected.

Expected Values

Panels of 40 serum as well as EDTA, citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) were tested for human IL-1α (see Table 3).
The levels measured may vary with the sample collection used. Elevated human IL-1α levels depend on the type of immunological disorder.
For detected human IL-1α levels see Table 3.

Table 3

Sample Matrix	Number of Samples Evaluated	Range (pg/mL)	% Detectable	Mean of Detectable (pg/mL)
Serum	40	nd*- 3.9	2.5	--
Plasma (EDTA)	40	nd*- 10.5	5.0	9.1
Plasma (Citrate)	40	nd*	--	--
Plasma (Heparin)	40	nd* - 51.7	17.5	13.7

* n.d. = non-detectable, sample measured below the lowest standard point are considered to be non-detectable.

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Reagent Preparation Summary

Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

Biotin-Conjugate

Make a 1:100 dilution of **Biotin-Conjugate** in Assay Buffer (1x):

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

Streptavidin-HRP

Make a 1:200 dilution of **Streptavidin-HRP** in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Human IL-1α Standard

Reconstitute lyophilized **human IL-1α standard** with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

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Test Protocol Summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.
   Alternatively external standard dilution in tubes (see 0): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µL Sample Diluent to sample wells.
6. Add 50 µL sample in duplicate, to designated sample wells.
7. Prepare Biotin-Conjugate.
8. Add 50 µL Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18°C to 25°C).
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 4 times with Wash Buffer.
12. Add 100 µL diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18°C to 25°C).
14. Empty and wash microwell strips 4 times with Wash Buffer.
15. Add 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
17. Add 100 µL Stop Solution to all wells.
18. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

USA: RUO

Revised 29 Dec. 2009 rm (Vers. 3)

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..