DRG® IL-1β ELISA (EIA-4437)

CE

RUO in the USA

REVISED 23 FEB. 2010 RM (VERS. 2.0)

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

1 INTENDED USE

Immunoenzymetric assay for the **in vitro** quantitative measurement of human Interleukin 1β (IL-1β) in serum and plasma.

2 CLINICAL BACKGROUND

2.1 Biological activities

Human interleukin-1 (IL-1) is a key mediator of the host response to various infectious, inflammatory and immunologic challenges. Two distinct polypeptides, IL-1α and IL-1 β, mediate IL-1 biological activities and bind to the same cell surface receptor. Both are initially synthesized as 31-kDA intracellular precursors that are subsequently found as mature proteins of 17 kDA in monocyte supernates. Membrane-bound IL-1 has also been described and may account for a part of IL-1 mediated local effects. The primary sources of IL-1 are blood monocytes and tissue macrophages. Other specialized cells such as T- and B-lymphocytes, various epithelial, endothelial and some mesenchymal cells can also produce IL-1. IL-1β is the major form secreted by monocytes and macrophages which are believed to be the main source of circulating (plasma) IL-1. Inhibitions of IL-1 activity have been described in plasma and other biological fluids. IL-1 affects several unrelated tissues and is a main mediator of the "acute phase" inflammatory responses characterised by alterations in metabolic, endocrinologic and immunologic functions. This cytokine has an essential role in T-cell activation, providing one of the necessary signals for IL-2 (T-cell growth factor) production. It is the main mediator of inflammatory processes by acting on the nervous system (fever, sleep, anorexia), on bone marrow-derived cells (chemotaxis and/or activation of neutrophils, monocytes and lymphocytes) and on various tissues (fibroblast proliferation, resorption of cartilage and bone matrices, glial cell proliferation, stimulation of endothelial cell procoagulant activity, etc.). Most of these activities are directly attributable to IL-1β, but others are mediated in collaboration with other cytokines such as IL-6, interferons, and tumor necrosis factor. IL-1 stimulates the production or acts synergistically with these cytokines and the final biological activity is thus the result of a network of interactions between these various mediators.

2.2 Clinical application

The biological properties of IL-1β and its key role in inflammatory processes suggest its involvement in the pathogenesis of many diseases. Indeed, high amounts of IL-1 are found in the joint effusions of some patients with rheumatoid and non-rheumatoid inflammatory joint diseases, in infectious pleural or peritoneal fluids, and in the drainage fluid of patients undergoing chronic diabetes, periodontal diseases, etc. Although little or no IL-1β is normally detected in human plasma or serum obtained from healthy, rested human subjects, elevated levels have been reported in the circulation of febrile or septic patients, in patients with Crohn's disease, during graft rejection, in healthy volunteers after extended exercise and in women following ovulation. Studies based on in vitro production of IL-1 by isolated blood leukocytes have demonstrated reduced IL-1 production in malnourished patients and cancer patients with large tumor burdens. Hence, this immunoassay for IL-1β is an important tool to study macrophage activation and to investigate the role of IL-1β in various (physiological or pathological) immune and inflammatory processes.

3 PRINCIPLES OF THE METHOD

The IL-1β ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-1β. Calibrators and samples react with the capture monoclonal antibody (MAb 1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human IL-1β – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-

labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the IL-1β concentration.
A calibration curve is plotted and IL-1β concentration in samples is determined by interpolation from the calibration curve.
The use of the EASIA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

4 REAGENTS PROVIDED

Reagents		96 tests Kit	Color Code	Reconstitution
Microtiterplate with 96 anti IL-1β (monoclonal antibodies) coated wells		96 wells	blue	**Ready** for use
Conjugate: HRP labelled anti- IL-1β (monoclonal antibodies) in TRIS-Maleate buffer with bovine serum albumin and thymol	**Ab HRP**	1 vial 6 ml	red	**Ready** for use
Calibrator N = 0 to 5 (see exact values on vial labels) in human serum, benzamidin and thymol	**CAL N**	6 vials lyophil.	yellow	**Add** 2.0 ml distilled water
Specimen Diluent: human serum, benzamidin and thymol	**DIL SPE**	3 vials lyophil	black	**Add** distilled water. (See on the label for the exact volume)
Wash Solution (Tris-HCl)	**WASH SOLN CONC**	1 vial 10 ml	brown	**Dilute** 200 x with distilled water (use a magnetic stirrer).
Controls - N = 1 or 2 in hum an serum, benzamidin and thymol	**CONTROL N**	2 vials yophil.	silver	**Add** 2.0 ml distilled water
Chromogen TMB (Tetramethylbenzydine) in Dimethylformamide	**CHROM TMB CONC**	1 vial 1 ml	green	**Dilute** 0.2 ml into 1 vial of substrate buffer
Substrate buffer: H_2O_2 in acetate / citrate buffer	**SUB BUF**	3 vials 21 ml	white	**Ready** for use
Stopping solution: H_2SO_4 1.8N	**STOP SOLN**	1 vial 6 ml	black	**Ready** for use

Note: 1. Use the specimen diluent for sample dilutions.
2. 1 pg of the calibrator preparation is equivalent to 100 mIU of the NIBSC 1st IS 86/680.

5 SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. High quality distilled water

2. Pipettes for delivery of: 50 µl, 200 µl, 1 ml and 10 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
6. Washer for Microtiterplates
7. Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (bichromatic reading)
8. Optional equipment: The ELISA-AID™ necessary to read the plate according to polychromatic reading (see paragraph 10.1.) can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

6 REAGENT PREPARATION

A. Calibrators:
Reconstitute the calibrators with 2.0 ml distilled water.

B. Controls:
Reconstitute the controls with 2.0 ml distilled water.

C. Specimen Diluent: Reconstitute the specimen diluent to the volume specified on the vial label with distilled water.

D. Working Wash solution:
Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize.
Discard unused Working Wash solution at the end of the day.

E. Revelation Solution:
Pipette 0.2 ml of the chromogen TMB into one of the vials of substrate buffer (H_2O_2 in acetate/citrate buffer).
Extemporaneous preparation is recommended.

7 STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators, controls and specimen diluent are stable for 4 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C for maximum 2 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

8 SPECIMEN COLLECTION AND PREPARATION

- Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4°C. If the samples are not used immediately, they must be kept at -20°C for maximum 2 months, and at -70°C for longer storage (maximum one year).
- Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Sampling conditions can affect values, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate IL-1β production by blood cells and thus falsely increase plasma IL-1β values.
- Collection tubes must be pyrogen-free. Plasma can be collected on sterile EDTA and rapidly separated after centrifugation. The use of heparin tubes is discouraged as batches of heparin are often contaminated with pyrogen.

9 PROCEDURE

9.1 Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.
Use a clean plastic container to prepare the Wash Solution.
In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
For the dispensing of the Revelation Solution and the Stop Solution avoid pipettes with metal parts.
High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section 11.5 (Time delay). Prepare a calibration curve for each run, do not use data from previous runs. The Revelation Solution should be colourless. If a blue colour develops within a few minutes after preparation, this indicates that the reagent is unusable, and must be discarded. Dispense the Revelation Solution within 15 minutes following the washing of the microtiterplate.
During incubation with Revelation Solution, avoid direct sunlight on the microtiterplate.

9.2 Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 200 µl of each Calibrator, Control and Sample into the appropriate wells.
4. Pipette 50 µl of anti- IL-1β-HRP conjugate into all the wells.
5. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
6. Aspirate the liquid from each well.

7. Wash the plate 3 times by:
   - Dispensing 0.4 ml of Wash Solution into each well
   - Aspirating the content of each well
8. Pipette 200 µl of the freshly prepared Revelation Solution into each well within 15 minutes following the washing step.
9. Incubate the microtiter plate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
10. Pipette 50 µl of Stop Solution into each well.
11. Read the absorbencies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 3 hours and calculate the results as described in section 10.

10 CALCULATION OF RESULTS

10.1 Polychromatic Reading:

a. In this case, the ELISA-AID™ software will do the data processing.
b. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
c. A second reading is performed at 490 nm against the same reference filter.
d. The ELISA-AID™ Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
e. The principle of polychromatic data processing is as follows:
   - Xi = OD at 450 nm
   - Yi = OD at 490 nm
   - Using a standard unweighted linear regression, the parameters A & B are calculated : Y = A*X + B
   - If Xi < 3 OD units, then X calculated = Xi
   - If Xi > 3 OD units, then X calculated = (Yi-B)/A
   - A 4 parameter logistic curve fitting is used to build up the calibration curve.
   - The IL-1β concentration in samples is determined by interpolation on the calibration curve.

10.2 Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. On semi-logarithmic or linear graph paper plot the OD values (ordinate) for each calibrator against the corresponding concentration of IL-1β (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

11 TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

IL-1β-ELISA		OD units Polychromatic model
Calibrator	0 pg/ml	0.013
	24 pg/ml	0.121
	89 pg/ml	0.336
	320 pg/ml	1.042
	574 pg/ml	1.693
	1166 pg/ml	2.704

12 PERFORMANCE AND LIMITATIONS

12.1 Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 0.35 pg/ml.

12.2 Specificity

No significant cross-reaction was observed in presence of 500 ng/ml of IL-1α, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, TNF-α, TNF-β , IFN-β , IFN-γ , TGF-β, GM-CSF, OSM , MIP-1α , MIP-1β , LIF, MCP-1, G-CSF, RANTES.
This IL-1β assay is specific for human natural and recombinant IL-1β.

12.3 Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	<X> ± SD (pg/ml)	CV (%)	Serum	N	<X> ± SD (pg/ml)	CV (%)
A	10	127 ± 3	2.3	A	20	120 ± 6	4.9
B	10	733 ± 11	1.4	B	20	549 ± 14	2.5

12.4 Accuracy

RECOVERY TEST

Sample	Added IL-1β (pg/ml)	Recovered IL-1β (pg/ml)	Recovery (%)
Serum	1282	1196	93
	605	542	90
	329	314	95
	157	131	84
	72	64	89
	31	28	92
Plasma	1282	1208	94
	605	573	95
	329	321	97
	157	146	93
	72	67	92
	31	29	94

DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)
Serum	1/1	-	1197
	1/2	598	637
	1/4	299	320
	1/8	150	164
	1/16	75	86
	1/32	37	41
Plasma	1/1	-	688
	1/2	344	336
	1/4	172	172
	1/8	86	87
	1/16	43	51
	1/32	26	22
	1/64	13	9
	1/128	4	4

Samples were diluted with specimen diluent.

12.5 Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrators have been added to the coated wells.

TIME DELAY				
	T0	10 min	20 min	30 min
1	1485	1575	1553	1647
2	1123	1129	1150	1228
4	592	572	595	606
5	375	391	375	375
6	1454	1429	1438	1605
500	641	583	645	658
1000	1107	1087	1158	1158
1500	1440	1261	1399	1425

12.6 Hook effect

A sample spiked with IL-1β up to 1 µg/ml gives higher OD's than the last calibrator point.

13 INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls that contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

14 REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

For guidance, the mean of 22 normal serum samples was 5.4 pg/ml (SD = 3.9), ranging between 0 pg/ml and 13.6 pg/ml. This study was performed on samples from apparently healthy persons with low CRP levels. For guidance, the mean of 103 normal plasma was 2.6 pg/ml (SD = 5.3), ranging between 0 pg/ml and 17 pg/ml (based on 2.5% to 97.5% percentiles). This study was performed with samples collected in strict sampling condition

15 PRECAUTIONS AND WARNINGS

Safety

For *in vitro* use only.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with all reagents, Stop Solution contains H_2SO_4, the chromogen contains TMB in Dimethylformamide, Substrate buffer contains H_2O_2. In case of contact, wash thoroughly with water.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

16 BIBLIOGRAPHY

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17 SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Calibrators (0-5)	200	-
Samples, Controls	-	200
Anti-IL-1β - HRP conjugate	50	50
Incubate for 2 hours at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Revelation Solution	200	200
Incubate for 15 min at room temperature with continuous shaking at 700 rpm.		
Stop Solution	50	50
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 (or 650 nm)		

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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 tilsttrækkeligt 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
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..