



### CE



Revised 2 Dec. 2009 (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-2 (IL-2) in serum.

#### 2 CLINICAL BACKGROUND

#### 2.1 Biological activities

IL-2, formerly called T-Cell growth factor, is a 14-16 kDA glycosylated polypeptide produced by activated CD4+ THcells which acts within an autocrine way to promote T-cells and NK-cells growth. T-cells respond to IL-2 via binding to the high affinity IL-2 receptor made up of three subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ). IL-2 can be found in culture supernatant after mitogen activation of mononuclear cells (PBMC) or T-clones.

#### 2.2 Clinical application

Low IL-2 concentration is detectable in serum/plasma of healthy donors, some data report elevated IL-2 levels in sera of systemic vasculitis and scleroderma patients.

#### **3** PRINCIPLES OF THE METHOD

The IL-2-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-2. 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-2 – 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-2 concentration.

A calibration curve is plotted and IL-2 concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA 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.





# ((



Revised 2 Dec. 2009 (Vers. 2.0)

#### **4 REAGENTS PROVIDED**

	Reagents	96 tests Kit	Color Code	Reconstitution
	<b>Microtiterplate</b> with 96 anti IL-2 (monoclonal antibodies) coated wells	96 wells	blue	Ready for use
Ab HRP	HRP Conjugate: HRP labelled anti-IL-2 (monoclonal antibodies) in TRIS-Maleate buffer with bovine serum albumin and thymol	1 vial 6 ml	red	<b>Ready</b> for use
CAL N	<b>Calibrator</b> $N = 0$ to 5 (see exact values on vial labels) in human serum with bovine serum albumin and thymol	6 vials lyophil.	yellow	Add 1 ml distilled water
DIL SPE	<b>Specimen Diluent:</b> human serum with bovine serum albumin, benzamidin and thymol	1 vial lyophil.	black	Add distilled water (see on the label for the exact volume)
INC BUFF	<b>Incubation Buffer:</b> Phosphate buffer with bovine serum albumin and thymol	1 vial 11 ml	black	Ready for use
WASH SOLN CONC	Wash Solution Concentrate (Tris-HCl)	1 vial 10 ml	brown	<b>Dilute</b> 200 x with distilled water (use a magnetic stirrer).
CONTROL N	<b>Control</b> - N = 1 or 2 in human serum with benzamidin and thymol	2 vials lyophil.	silver	Add 1 ml distilled water
CHROM TMB	TMB Substrate Solution Chromogenic TMB Solution	1 vial 25 ml	white	Ready for use
STOP SOLN	<b>Stop Solution:</b> HCl, 2N	1 vial 25 ml	white	Ready for use

Note: 1. Use Specimen Diluent for sample dilutions.

2. 1 U of the calibrator preparation is equivalent to 1 U of the NIBSC 1<sup>st</sup> IS 86/504.

#### **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, 100 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





# CE



#### Revised 2 Dec. 2009 (Vers. 2.0)

- 5. Horizontal microtiter plate shaker capable of 700 rpm  $\pm$  100 rpm
- 6. Washer for Microtiter plates
- 7. Microtiter plate 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<sup>™</sup> 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 1 ml distilled water.

#### B. Controls:

Reconstitute the controls with 1 ml distilled water.

#### C. Specimen Diluent:

Reconstitute 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.

#### 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.
- 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 -70°C for maximum 1 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.





# CE



#### Revised 2 Dec. 2009 (Vers. 2.0)

- 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-2 production by blood cells and thus falsely increase serum IL-2 values.
- Collection tubes must be pyrogen-free.

#### 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 Chromogenic 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 12.5 (Time delay).
- Prepare a calibration curve for each run, do not use data from previous runs.
- Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
- During incubation with Chromogenic 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 100 µl of Incubation Buffer into all the wells
- 4. Pipette 100 µl of each Calibrator, Control and Sample into the appropriate wells.
- 5. Pipette 50 µl of anti-IL-2-HRP conjugate into all the wells.
- 6. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm  $\pm$  100 rpm.
- 7. Aspirate the liquid from each well.
- Wash the plate 3 times by: Dispensing 0.4 ml of Wash Solution into each well Aspirating the content of each well
- 9. Pipette 100 µl of the chromogenic solution into each well within 15 minutes following the washing step.

#### DRG International, Inc., USA Fax: (908) 233 0758 e-mail: corp@drg-international.com





# CE



#### Revised 2 Dec. 2009 (Vers. 2.0)

- 10. Incubate the microtiterplate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm  $\pm$  100 rpm, avoid direct sunlight.
- 11. Pipette 200 µl of Stop solution into each well.
- 12. 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

- 1. In this case, the ELISA-AID<sup>™</sup> software will do the data processing.
- 2. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
- 3. A second reading is performed at 490 nm against the same reference filter.
- 4. The ELISA-AID<sup>™</sup> Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
- 5. The principle of polychromatic data processing is as follows:

Xi = OD at 450 nm

Yi = OD at 490 nm

Using a calibrator 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-2 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-2 (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.





### CE



Revised 2 Dec. 2009 (Vers. 2.0)

#### 11 TYPICAL DATA

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

IL-2-ELISA			OD units Polychromatic model
	Standard 0 U/n	nl	0.026
	0.78 U/n	nl	0.069
	1.77 U/n	nl	0.144
	5.7 U/n	nl	0.650
	10.9 U/m	nl	1.511
	23.8 U/m	nl	3.667

#### **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 calibrator deviations above the average OD at zero binding, was 0.05 U/ml.

#### 12.2 Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1 $\alpha$ , IL-1 $\beta$ , IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, TNF- $\alpha$ , TNF- $\beta$ , IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , TGF- $\beta$ , GM-CSF, OSM, MIP-1 $\alpha$ , MIP-1 $\beta$ , LIF, MCP-1, G-CSF and RANTES. This IL-2 assay is specific for human natural and recombinant IL-2.

#### Interference with the soluble IL-2 receptor (sCD-25)

To check the absence of any interference of the sR-IL-2 (sCD-25) on the assay, different sCD-25 concentrations were spiked in the calibrators.





### RUO IN THE USA

#### Revised 2 Dec. 2009 (Vers. 2.0)

IL-2 calibrators		+ CD25	0.1 ng/ml	+ CD25	1 ng/ml	+ CD25	10 ng/ml
(U/ml)	mOD	m	OD	m(	DD	m	OD
0	12		14		19		15
1	92		88		77		84
2.5	211		198		214		196
7.5	904		796		909		647
15	1878		1855		2002		2080
30	4260		3708		3785		3649

#### 12.3 Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	$\pm$ SD (U/ml)	CV (%)	Serum	N	$\pm$ SD (U/ml)	CV (%)
А	20	$3.2 \pm 0.1$	3.2	А	22	$3.9 \pm 0.2$	5.1
В	20	$9.6 \pm 0.2$	2.2	В	22	$9.6 \pm 0.3$	3.1

SD: Standard Deviation; CV: Coefficient of variation

#### 12.4 Accuracy

**RECOVERY TEST** 

Sample Added IL-2 (U/ml)		Recovered IL-2 (U/ml)	Recovery (%)
	0	0	
	1.9	1.8	95
Comuna 1	2.8	2.9	104
Serum I	9.7	9.1	94
	18.4	17.8	97
	0	0	
	1.9	1.9	100
Somum 2	2.8	2.8	100
Serum 2	9.7	9.3	96
	18.4	17.2	93







#### Revised 2 Dec. 2009 (Vers. 2.0)

#### DILUTION TEST

Sample Dilution		Theoretical Concent. (U/ml)	Measured Concent. (U/ml)	
	1/1	-	21.7	
	1/2	10.8	9.9	
Samu	1/4	4.9	5.1	
Serum	1/8	2.6	3.0	
	1/16	1.5	1.4	
	1/32	0.7	0.6	
	1/64	0.3	0.3	
	1/128	0.2	0.2	

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.

Sample	0 min	10 min	20 min	30 min	40 min
1	1.6	1.4	1.5	1.4	1.5
2	3.9	3.6	3.7	3.7	3.7
3	7.6	7.3	7.1	7.0	7.7
4	16.8	15.9	16.2	16.4	16.8

#### 12.6 Hook effect

A sample spiked with IL-2 up to 500 U/ml gives higher OD's than the last calibrator point.





# CE



Revised 2 Dec. 2009 (Vers. 2.0)

#### **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 results of 40 serum samples from apparently healthy persons with low CRP levels, ranged between 0 and 0.1 U/ml.

38 samples obtained values below the detection limit of the test (<0.05 U/ml).

#### **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 HCl. 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.





### CE



Revised 2 Dec. 2009 (Vers. 2.0)

#### **16 BIBLIOGRAPHY**

1. ROBB R.J., (1984). Interleukin-2 : The molecule and its function. Immunology. Today, 5:203.

- GRAU G. et al, (1989).
  Serum Cytokine changes in systemic vasculitis. Immumnology, 68:196-198.
- 3. BASCHAR M. et al, (1989). I nterleukin-2 in Sclerodemia : Correlation of serum level with extent of skin involvement and disease duration. An. Internal Med., 110:446-450.

#### **17 SUMMARY OF THE PROTOCOL**

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)				
Incubation buffer Calibrators (0-5)	100 100	100				
Samples, Controls Anti-IL-2 -HRP conjugate	- 50	100 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.						
Chromogenic Solution	100	100				
Incubate for 15 min at room temperature with continuous shaking at 700 rpm.						
Stop Solution	200	200				
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 (or 650						







#### Revised 2 Dec. 2009 (Vers. 2.0)

#### SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
[]i	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
T	Contains sufficient for <n> tests/</n>	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos</n>	Contenuto sufficiente per "n" saggi
1	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
$\Sigma$	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	
<b>l</b>	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	
T		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
1	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης	
$\Sigma$	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	