Before use, read this Package Insert.

IL-6 - IRMA

An immunoradiometric assay kit for the Interleukin 6 in serum, plasma and culture media.
For Research Use only

I GENERAL INFORMATION

A. Proprietary Name : DIAsource IL-6 IRMA kit

B. Catalogue Number : KIC1261 : 96 determinations

C. Manufactured by : DIAsource ImmunoAssays S.A.
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II APPLICATION AND INTENDED USE

Human Interleukin 6 (IL-6) is a 184 A.A. polypeptide with potential O and N-glycosylation sites, and a significant homology with G-CSF. It is produced by various cells, including T and B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. It regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoïesis, and inflammation. These multiple actions are integrated within a complex cytokines network, where several cytokines are induced (IL-1, TNF, PDGF, IFNs, …). The final effects result from either synergistic or antagonistic activities between IL-6 and the other cytolines (IL-1, IL-2, IL-3, IL-4, IL-5, IFN-γ, G-CSF, M-CSF, CSF, …). IL-6 induces final maturation of B cells into antibody producing cells and is a potent growth factor for myeloma/plasmacytoma cells. It (co-)stimulates T cell growth and cytoxic T cell differentiation. It promotes megakaryocyte development and synergizes with other cytokines to stimulate multipotent hematopoïetic progenitors. It can also induce differentiation and growth inhibition of some leukemia – or non hematopoïeteic tumoral – cell lines. IL-6 is also a major inducer of the acute phase reactions in response to inflammation or tissue injury. Along with IL-1 and TNF, it induces the synthesis of acute phase proteins (APP) by hepatocytes, each cytokine or combination of cytokines showing a preferential pattern of APP production. IL-6 also interacts with the neuroendocrine system, e.g. by inducing ACTH production. Thus IL-6 is a pleiotropic cytokine with multiple endocrine, paracrine and possibly autocrine activities in various tissues.
Although most normal controls have indetectable level of IL-6 in their serum, huge quantities of IL-6 are detected in severe inflammatory situations such as septicemia. The elevation of serum IL-6 precedes that of acute phase proteins, e.g. in a postoperative phenomenon, and may thus be a sensitive early parameter to investigate in inflammatory conditions. Serum IL-6 has already been described in association with surgical or traumatic tissue injuries, infectious diseases, autoimmune diseases including arthritis, graft rejection, alcoholic liver cirrhosis, malignancies, etc.

III PRINCIPLES OF THE DIAsource IL-6 IRMA ASSAY

The DIAsource IL-6 IRMA assay is a two-step immunoradiometric assay based on coated-tube separation. Mabs 1 – the capture antibodies – are attached to the lower and inner surface of the plastic tube. Standards or samples are added to the tubes. After incubation, washing removes the occasional excess of antigen. Mab 2 – the 125 I-labelled-antibody is added. After incubation and washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyper specificity – common to two-site IRMA, as well as the need of a shaker or incubation at 37°C
Because of the occasional extremely high concentration of IL-6 in serum, the present DIAsource IL-6 IRMA has been developed on a two steps procedure to avoid high dose hook effects.

IV REAGENTS PROVIDED

1. Reagents

Reagents	Quantity	Colour	Reconstitution Code
Anti-IL-6 coated tubes (monoclonal antibodies)	2 x 48	melon	**Ready** to use
Anti-IL-6-I-125 (Monoclonal antibodies in phosphate buffer with bovine serum albumin and azide)	2 vials 2 x 10.5 ml 2 x 220 KBq	red	**Ready** to use
Diluent in plasma and merthiolate	1 vial 8 ml lyophilized	black	**Add** 8 ml distilled water
Standards 0-5 (see exact value on the vials labels) in plasma and merthiolate	6 vials 2 ml lyophilized	yellow	**Add** 2 ml distilled water
Controls 1 and 2 in human plasma and merthiolate	2 vials 2 ml lyophilized	silver	**Add** 2 ml distilled water
Buffer	2 vials 11 ml	green	**Ready** to use
Washing Solution	1 vial 10 ml	brown	**Dilute** the content in 700 ml distilled water (use a magnetic stirrer)

Note: 1) use the content of the diluent for plasma, serum and culture media dilutions.
2) 1 pg = 3.50 mIU MRC 88/514
1 pg = 100 mIU MRC 89/548

2. Storage and stability
Store all kit reagents until the expiry date and any residual solution up to 4 days at 4°C after reconstitution.
For prolonged storage standards and controls must be kept at -30°C.

V EQUIPMENT AND SUPPLIES REQUIRED BUT NOT PROVIDED

1. Distilled water
2. Pipettes : 50 µl, 200 µl, and 2 ml (the use of accurate micropipettes with disposable plastric tips is recommended)
3. 5 ml automatic syringe (Comwall type) for washing
4. Water aspiration system (optional)
5. Gamma counter (I-125), vortex mixer and magnetic stirrer

VI SPECIMEN COLLECTION AND PREPARATION

IL-6 may be assayed in serum, plasma, other biological fluids, or supernatants from in vitro cultured cells.
For serum or plasma IL-6 assay, very strict precautions have to be taken during sampling, to avoid impurities contained in sampling materials that would stimulate IL-6 production by blood cells and thus falsely increase plasma IL-6 values (e.g. various commercial heparinised tubes contain endotoxins that stimulate IL-6 production).
Therefore, it is recommended :
- either to utilize serum collected on sterile, clean, dry tubes, and rapidly separated after coagulation
- either to utilize plasma rapidly separated from sample collected in EDTA sterile tube. Heparin and other substances non-tested in tubes should be avoided, as well as prolonged delay before cellular phase separation. Samples must be kept frozen (- 20°C) if assaying is not done immediately.
- Some pathologic sera or plasma (severe inflammatory situation, cirrhosis, etc) can contain very high concentrations of IL-6 as well as culture media of PBMC after PHA or LPS activation. It is advisable to dilute those high IL-6 samples with the plasma diluent supplied with the kit.

VII ASSAY PROCEDURE

Adherence to the instructions are mandatory to obtain reliable results. Avoid to mix different lots of coated tubes.
1. Preparation of reagents : first reconstitute standards and controls with 2 ml distilled water.
2. Label coated tubes in duplicate for each standard, sample and control. For determination of total counts, label 2 normal tubes.
3. Vortex standards, samples, controls and dispense 200 µl of each into respective tubes.
4. Dispense 200 µl of buffer in all the tubes.
5. Shake gently and manually tube rack.
6. Incubate 16-20 hours at room temperature.
Remark : the temperature should not exceed 30°C
7. Aspirate (or decant) the complete content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
8. Wash tubes with 2 ml washing solution and aspirate (or decant). Avoid foam during the addition of the washing solution.
9. In order to increase the reproducibility of the assay, leave the tubes on the table for two minutes and aspirate the remaining drop of liquid.
10. Dispense 200 µl tracer in each tube, including total counts.
11. Shake gently and manually tube rack.
12. Incubate for 2 hours at room temperature.
Remark : the temperature should not exceed 30°C.
13. Aspirate (or decant) the complete content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
14. Wash tubes with 2 ml washing solution and aspirate (or decant). Avoid foam during the addition of the washing solution.
15. In order to increase the reproducibility of the assay, leave the tubes on the table for two minutes and aspirate the remaining drop of liquid.
16. Count tubes in gamma counter for 60 seconds.
17. The standard curve is prepared by plotting cpm or B/T (%) on the ordinate against the standard concentration on the abscissa using either linear-linear semi-log graph paper. Draw the best curve rejecting obvious outlyers. Exam of standard curves are included (see table : examples of typical reference curve). The data are presented as an example only and should not be used instead of the standard curve prepared by the analyst for each assay.
18. Determine IL-6 concentrations of samples by interpolation of cpm or B/T (%) values. If a computer assisted method is used, a polynomial function usually gives the best results.

SUMMARY OF ASSAY PROCEDURE			
	Total Counts ml	Standards ml	Sample(s) ml
Standards (0-5)	-	0.200	-
Samples	-	-	0.200
Buffer	-	0.200	0.200
Incubation	16-20 hours at room temperature		
Separation	-	Aspirate (or decant)	
Washing solution	-	2.0	2.0
Separation	-	Aspirate (or decant)	
Tracer	0.2	0.2	0.2
Incubation	2 hours at room temperature		
Separation	-	Aspirate (or decant)	
Washing solution	-	2.0	2.0
Separation	-	Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

EXAMPLE OF TYPICAL REFERENCE CURVE			
IL-6 IRMA		CPM	B/T x 100
Total count		145869	
Std	0 pg/ml	212	0.2
	50 pg/ml	703	0.5
	150 pg/ml	1603	1.1
	500 pg/ml	4613	3.2
	1500 pg/ml	11532	7.9
	5000 pg/ml	30442	20.9

VIII EXPECTED VALUES

At the present stage of the study, only preliminary results can be provided. 157 normal sera were evaluated in this assay : 39 had non-detectable levels of IL-6, the other sera displayed low but detectable values (range 6-31 pg/ml).

IX PERFORMANCE CHARACTERISTICS

1. Minimum detectable concentration (MDC)

The MDC is estimated to be 6 pg/ml and is defined as the IL-6 concentration corresponding to the mean CPM of 20 replicates of the zero standard + 2 standard deviations.

2. Specificity

The cross-reactivity was determined by addition of different analytes to 0, 65 or 600 pg/ml of IL-6 and the apparent IL-6 concentration was measured.

Added analyte to IL-6 samples		Obser. values for 0 pg/ml of IL-6	Observ. values for 65 pg/ml of IL-6	Observ. values for 600 pg/ml of IL-6
IL-1α	50 ng/ml	< 3	68.9	590.3
IL-1β	50 ng/ml	< 3	64.5	604.1
IL-2	50 ng/ml	<3	66.3	618.2
IL-3	50 ng/ml	<3	64.3	586.1
IL-4	50 ng/ml	<3	64.0	591.7
GM-CSF	50 ng/ml	<3	62.6	624.6
G-CSF	50 ng/ml	29.6	97.4	584.6
IFN-α	50 ng/ml	< 3	64.2	591.6
IFN-γ	50 ng/ml	< 3	63.3	584.2
TNF-α	50 ng/ml	< 3	60.0	598.1
TNF-β	50 ng/ml	< 3	61.9	593.6

This demonstrates that IL-6 Irma does not crossreact with IL-1α, IL- β, IL-2, IL-3, IL-4, GM-CSF, IFN-α, IFN-γ, TNF-α and TNF-β. A very tenuous cross-reaction is observed for G-CSF.

3. Precision

Serum	N	<X> ± SD	CV %	Serum	N	<X> ± SD	CV %
A	20	277 ± 12	4.3	A	10	282 ± 6.5	2.3
B	20	893 ± 47	5.3	B	10	970 ± 44	4.5
C	20	1576 ± 106	6.7	C	10	1739 ± 145	8.3

4. Accuracy

Recovery Test

Sample	Added pg/ml	Recovered g/ml	Recovery (%)
Serum	3230	3197	99
	1981	1703	86
	826	718	87
	367	328	89
Plasma	3230	3131	97
	1981	1935	98
	826	653	79
	367	311	85
Culture media	3230	3366	104
	1981	1674	85
	826	883	107
	367	370	101

Dilution Test

Sample	Theoretical concent.	Measured concent.	Recovery (%)
Serum			
1/1	1287	-	-
1/2	644	744	115
1/4	322	397	123
1/8	161	204	126
1/16	80.5	88	109
Plasma			
1/1	1467	-	-
1/2	734	700	95
1/4	367	368	100
1/8	184	198	108
1/16	92	113	123
Culture media			
1/1	3487	-	-
1/2	1744	1554	89
1/4	872	796	91
1/8	436	377	86
1/16	218	229	105

X WARNINGS

1. For Research Use only.
2. This kit contains ^{125}I (half-life : 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
3. Human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, 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, e.g. CDC/HIH Health Manual : "Biosafety in Microbiological and Biomedical Laboratories" 1984.
4. Certain reagents contain azide as preservative. When dry, azide can be explosive. Therefore it is advisable to rinse the sink with running water when decanting solutions which contain azide.
5. Adherence to the basic rules of radiation safety should provide adequate protection. See National Bureau of Standard Handbooks 92, "Safe Handling of Radioactive Materials", March 9, 1964, Washington D.C. A summary follows :
   - do not eat, drink, smoke or apply cosmetics where radioactive materials are used;
   - do not pipet radioactive solutions by mouth;
   - avoid direct contact with all radioactive materials by using protective clothing such as lab coats and disposable gloves;
   - all radiological work should be done in a designated area away from traffic;
   - radioactive materials should be stored in their original containers in a designated area;
   - a record book for logging receipt and disposal of all radioactive materials should be kept;
   - laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes;
   - any radioactive spills should be taken care of immediately, in accordance with established procedures;
   - all reactive materials must be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.

XI BIBLIOGRAPHY

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