Interleukin-6 (IL-6) (Human) ELISA Kit

BGT-KET-260

96Wells

REF BGT-KET-260

2°C–8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

Interleukin-6 (IL-6) (Human) ELISA Kit

1. INTENDED USE

Immunoenzymetric assay for the measurement of human interleukin-6 (IL-6) in serum.

2. BACKGROUND

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 cytokine network, where several cytokines induce (IL-1, TNF, PDGF, IFNs,...) or are induced by IL-6 and the final effects result from either synergistic or antagonistic activities between IL-6 and the other cytokines (IL-1, IL-2, IL-4, IL-5,IFNγ, IL-3, GM-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 cytotoxic 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 hematopoïetic 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.

3. PRINCIPLES OF THE METHOD

The IL-6-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of IL-6. 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-6 – 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-6 concentration.
A calibration curve is plotted and IL-6 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.

4. REAGENTS PROVIDED

96	**Microtiterplate** - **Ready** for use
Contents:	Microtiterplate with 96 anti-IL-6 (monoclonal antibodies) coated wells
Color Code:	blue
CONJUGATE	**Conjugate** - **Ready** for use
Contents:	HRP labelled anti-IL-6 (monoclonal antibodies) in Borate buffer with bovine serum albumin and thymol
Volume:	1 x 11 ml
Color Code:	red

Calibrators and **Controls -** lyophilized

Symbol	Calibrator / Control
CAL 0	**Calibrator 0**
CAL 1	**Calibrator 1**
CAL 2	**Calibrator 2**
CAL 3	**Calibrator 3**
CAL 4	**Calibrator 4**
CAL 5	**Calibrator 5**
CONTROL 1	**Control 1**
CONTROL 2	**Control 2**

Contents: **(See exact values on vial label)** in human plasma with bovine serum albumin, benzamidin and thymol

Preparation: **Add** 1 ml distilled water

Color Code: Calibrator yellow
Control silver

DILUENT **Specimen Diluent** - lyophilized

Contents: human plasma with bovine serum albumin, benzamidin and thymol

Volume: 2 vials

Preparation **Add** distilled water (see on the label for the exact volume)

Color Code: black

INC-BUFF **Incubation Buffer** - **Ready** for use

Contents: Borate buffer with bovine serum albumin, benzamidin and thymol

Volume: 1 x 11 ml

Color Code: black

WASH-CONC 200x **Wash Solution** - 200x concentrated

Contents: Wash Solution (TRIS-HCl)

Volume: 1 x 10 ml

Preparation: **Dilute** 200x with distilled water (use a magnetic stirrer).

Color Code: brown

SUBSTRATE **Chromogenic TMB Solution** - **Ready** for use

Contents: Chromogenic TMB Solution

Volume: 1 x 25 ml

Color Code: white

STOP-SOLN **Stop Solution** - **Ready** for use

Contents: 2N HCl

Volume: 1 x 12 ml

Color Code: white

Hazards identification:

H314 Causes severe skin burns and eye damage.

Note:

1. Use the *Specimen Diluent* for sample dilution.
2. 1 pg of the calibrator preparation is equivalent to 100 mIU of the NIBSC 1st IS 89/548.

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
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)

6. REAGENT PREPARATION

Calibrators:
Reconstitute the calibrators with 1 ml distilled water.

Controls:
Reconstitute the controls with 1 ml distilled water.

Specimen Diluent:
Reconstitute *Specimen Diluent* to the volume specified on the vial label with distilled water.

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 (18 - 25 °C) 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 -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 (18 - 25 °C). 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-6 production by blood cells and thus falsely increase serum IL-6 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 (18 - 25 °C) 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.
- Each well can only be used once.

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 50 µl of Incubation Buffer into all the wells.
4.	Pipette 100 µl of each Calibrator, Control and Sample into the appropriate wells.
5.	Incubate for 1 hour at room temperature (18 - 25 °C) 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 100 µl of anti-IL-6-HRP conjugate and 50 µl specimen diluent into all the wells.
9.	Incubate for 1 hour at room temperature (18-25°C) on a horizontal shaker set at 700 rpm ± 100 rpm.
10.	Aspirate the liquid from each well.
11.	Wash the plate 3 times by: • Dispensing 0.4 ml of Wash Solution into each well • Aspirating the content of each well
12.	Pipette 200 µl of the Chromogenic Solution into each well within 15 minutes following the washing step.
13.	Incubate the microtiterplate for 15 minutes at room temperature (18 - 25 °C) on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
14.	Pipette 100 µl of Stop Solution into each well.
15.	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, 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 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 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-6 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. Plot the OD values (ordinate) for each calibrator against the corresponding concentration of IL-6 (abscissa) and draw a calibrator curve through the calibrator points.
4. Read the concentration for each control and sample by interpolation on the calibrator 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 calibrator curve.

IL-6-ELISA		OD units Polychromatic model
Calibrator	0 pg/ml	79
	23.3 pg/ml	125
	68 pg/ml	193
	201 pg/ml	408
	633 pg/ml	1036
	2560 pg/ml	3579

12. PERFORMANCE AND LIMITATIONS

12.1 Detection Limit

Twenty Calibrator 0 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 2 pg/ml.

12.2 Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, GM-CSF, IFN-α, IFN-γ, LIF, MIP-1α, MIP-1β, MCP-1, OSM, RANTES, TGF-β, TNF-α and TNF-β. A very tenuous cross-reaction (0.06%) is observed with G-CSF.

Interference with the soluble Receptors (sIL6R and sgp-130)
No significant cross-reaction was observed in presence of 100 ng of sIL6 Receptor and sgp-130.

IL-6 conc. (pg/ml)	IL-6 measured with 100 ng/ml of sIL6R (pg/ml)	IL-6 measured with 100 ng/ml of sgp-130 (pg/ml)
7.5	4.3	8.3
74.0	81.8	76.0
678.0	734.0	671.0

No interference was observed.

12.3 Precision

Intra Assay				Inter Assay			
Serum	N	<X> ± SD (pg/ml)	CV (%)	Serum	N	<X> ± SD (pg/ml)	CV (%)
A	20	147 ± 6.1	4.2	A	20	114 ± 5	4.4
B	20	623 ± 27	4.3	B	20	270 ± 15	5.4

SD: Standard Deviation; CV: Coefficient of variation

12.4 Accuracy

Recovery Test

Sample	Added IL-6 (pg/ml)	Recovered IL-6 (pg/ml)	Recovery (%)
Serum 1	1066 547 228	1035 541 234	97.1 98.9 102.6
Serum 2	1066 547 228	1110 531 250	104.1 97.1 109.6

Dilution Test

Sample	Dilution	Theoretical Conc. (pg/ml)	Measured Conc. (pg/ml)
Serum	1/1	-	966
	1/2	483	478
	1/4	241.5	247
	1/8	120.8	130
	1/16	60.4	54
	1/32	30	23

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

Sample	0 min	10 min	20 min	30 min	40 min
1	61	53	56	61	76
2	196	179	205	213	273
3	1584	1478	1433	1418	1533

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 34 serum samples from apparently healthy persons with low CRP levels, ranged between 0 and 50 pg/ml. 31 samples obtained values below 17 pg/ml.

15. PRECAUTIONS AND WARNINGS

Safety

For research 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.
For more information, see Safety Data Sheet (SDS).

16. BIBLIOGRAPHY

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17. SUMMARY OF THE PROTOCOL

	Calibrator (µl)	Sample(s) / Controls (µl)
Incubation Buffer Calibrators (0 - 5) Samples, Controls	50 100 -	50 - 100
Incubate for 1 hour at room temperature (18 - 25 °C) with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Anti-IL-6 -HRP conjugate Specimen Diluent	100 50	100 50
Incubate for 1 hour at room temperature (18 - 25 °C) 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	200	200
Incubate for 15 min at room temperature (18 - 25 °C) with continuous shaking at 700 rpm.		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (and 490 nm) versus 630 (or 650 nm)		

The manufacturer can offer you the possibility of acquiring a protocol adapted for this kit to be used on the Stratec Gemini 2PS + Combo platform including protocol assay file, reagent file and tips for the good use of the kit on the instrument.