III PRINCIPLES OF THE BIOSOURCE IL-17 CYTOSCREEN ASSAY

The BIOSOURCE IL-17 Cytoscreen is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiter plate. Standards or samples containing IL-17 react with capture monoclonal antibody (Mab 1) coated on the microtiter well and with a biotinylated monoclonal antibody (Mab 2). After an incubation period allowing the formation of a sandwich : coated Mab 1 - IL-17 - Mab 2 - Biotin, the microtiter plate is washed to remove unbound biotinylated antibodies. Streptavidin-Peroxidase is added and this binds to the biotinylated antibody. After incubation, the unbound enzyme is removed by washing and a substrate solution is added. The reaction is stopped with the addition of Stop Solution and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the IL-17 concentration. A standard curve is plotted and IL-17 concentrations in a sample is determined by interpolation from the standard curve.

IV REAGENTS PROVIDED

Reagents	96 tests Kit	192 tests Kit	Reconstitution
Microtiter plate with 96 anti-IL-17 coated wells	1 x 96 wells	2 x 96 wells	**Ready** for use
Standard in bovine plasma with preservatives: see vial label for exact concentration	3 vials lyophil.	3 vials lyophil.	**Reconstitute** with distilled water to the volume specified on the vial label
Solution B (buffer with preservatives)	1 vial 22 ml	1 vial 22 ml	**Ready** for use
Solution A (human plasma with preservatives)	3 vials lyophil.	4 vials lyophil.	**Add** distilled water (see the volume on the vial label)
Anti-IL-17-Biotin Conjugate in buffered solution with proteins and preservatives	1 vial 6 ml	2 vials 6 ml	**Ready** for use
Streptavidin-HRP diluent with preservatives	1 vial 22 ml	1 vial 22 ml	**Ready** for use
Controls 1 and 2 in human plasma with preservatives	2 vials lyophil.	2 vials lyophil.	**Add** 2 ml distilled water
Washing Solution Concentrate (buffer with preservatives)	1 vial 10 ml	1 vial 10 ml	**Dilute** 2 ml in 400 ml distilled water or the vial content in 2000 ml distilled water
Concentrated Streptavidin-HRP	1 vial 0.3 ml	2 vials 0.3 ml	**See** the table (VII.3)
Chromogen : TMB	1 vial 25 ml	1 vial 25 ml	**Ready** for use
Stop Solution	1 vial 25 ml	1 vial 25 ml	**Ready** for use

V PRECAUTIONS AND WARNINGS

1. The human blood components included in this kit have been tested by European approved and USA FDA approved methods and found negative for HBsAg, anti-HCV and 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.
2. Avoid any skin contact with Stop Solution and Chromogen (TMB). In case of contact wash thoroughly with water.
3. Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
4. Do not pipet liquids by mouth.
5. Bovine material used in this kit originates from animals coming from countries where BSE has not been reported.

I EQUIPMENT AND SUPPLIES REQUIRED BUT NOT PROVIDED

1. High quality distilled water.
2. Precision pipette : 50 µl, 100 µl, 250 µl, 1 ml and 10 ml.
3. Vortex mixer and magnetic stirrer.
4. Horizontal microtiter plate shaker capable of 700 rpm ± 100 rpm, microtiter plate reader fitted out with 450, 405 and 650 or 630 nm filter, microtiter plate washer.

VII REAGENT PREPARATION

1. **Solution A and Controls** : Reconstitute the lyophilized Solution A, and Controls to the volume specified on the vial label with distilled water. Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion.
2. **Standard**: Reconstitute the lyophilized standard to 2500 pg/ml with distilled water. Refer to standard vial label for instructions. Swirl or mix gently to ensure complete reconstitution. Make serial dilutions of the standard as described in the following table

Standard		Add	Into Solution A
1000	pg/ml	200 µl of std 2500 pg/ml	300 µl
500	pg/ml	250 µl of std 1000 pg/ml	250 µl
250	pg/ml	250 µl of std 500 pg/ml	250 µl
125	pg/ml	250 µl of std 250 pg/ml	250 µl
62.5	pg/ml	250 µl of std 125 pg/ml	250 µl
31.3	pg/ml	250 µl of std 62.5 pg/ml	250 µl
15.6	pg/ml	250 µl of std 31.3 pg/ml	250 µl
0	pg/ml		250 µl

3. **Streptavidin-HRP Dilution (51 x concentrated)** : Following the number of wells to be used, dilute the concentrated conjugate with the Streptavidin-HRP diluent in a clean glass vial: see below table for the volumes to pipette. Extemporaneous preparation is recommended.

TABLE STREPTAVIDIN-HRP DILUTION

Number of wells	Streptavidin-HRP	Streptavidin conjugate diluent
16	40 µl	2 ml
32	80 µl	4 ml
48	120 µl	6 ml
96	220 µl	11 ml
192	440 µl	22 ml

4. **Wash Solution** : Dilute 2 ml of Washing Solution Concentrate in 400 ml distilled water or all the content of the Washing Solution Concentrate vial in 2000 ml distilled water (use a magnetic stirrer).

VIII STORAGE AND SHELF LIFE OF REAGENTS

A. UNOPENED vials

Store the unopened vials at 2˚C to 8˚C. All kit components are stable until the expiry date printed on the labels.

B. OPENED vials

1. The Biotin Conjugate vial must be stored at 2˚ to 8˚C.
2. The reconstituted Standards, and Solution A are stable for 4 days at 2˚C to 8˚C. Aliquots held for longer periods of time should be frozen, a maximum of two times, at -20˚C (maximum 2 months) or at -70˚C for longer storage (until expiration date).
3. The reconstituted controls must be frozen immediately after use. They can be frozen a maximum of three times and must be stored at -20˚C (maximum 1 month) or at -70˚C for longer storage.
4. Store the unused Streptavidin-HRP at 4˚C until expiration date of the kit.
5. Store the unused strips at 2˚C to 8˚C in the sealed bag containing the desiccant until expiration date.
6. The Washing Solution Concentrate is stable at room temperature until expiration date. In order to avoid washerhead obstructions, it is recommended to prepare a fresh diluted Wash Solution each day.

X SPECIMEN COLLECTION, PREPARATION, STORAGE AND DILUTION

A. Specimen Collection and preparation

1. The BIOSOURCE IL-17 Cytoscreen kit may be used to measure IL-17 in serum, plasma and cell culture supernatant .Isolation and culture of peripheral blood mononuclear cells may be realized by usual methods. However, one should avoid an unintentional stimulation of the cells by the procedure. The use of pyrogen-free reagents and adequate controls are mandatory.
2. Sampling conditions can affect values measured in serum or plasma, therefore, strict precautions have to be taken during sampling to avoid impurities contained in sampling materials that would stimulate IL-17 production by blood cells and thus falsely increase plasma IL-17 values.
3. Serum must be removed as soon as possible from the clot of red cells after clotting and centrifugation, and kept at 4˚C for maximum one day.
4. Collection tubes must be pyrogen-free. Plasma can be collected on sterile EDTA or heparin tubes (at 4˚C) and rapidly separated after centrifugation. However, as batches of heparin are often contaminated with pyrogen, it is recommended to test each batch of heparin to avoid unintentional stimulation of blood cells. Other substances in the tube must be also pyrogen-free.

B. Storage

Serum/plasma samples must be kept at -20˚C for maximum 2 months, and for longer storage (maximum one year) at - 70˚C. Samples with low protein levels (e.g. cell culture medium,) should be stored at -70˚C (maximum one year).

C. Sample Dilution

If samples generate values higher than the last standard point, dilute the sample with the appropriate solution (see below).

1 **Serum and plasma** : dilute with Solution A.
2. **Cell culture supernatant** : dilute with Solution B or corresponding medium.

X BIOSOURCE IL-17 CYTOSCREEN PROCEDURE

The instructions of the assay procedure must be followed to obtain reliable results.

A. Procedural notes

1. Allow the samples and reagents to equilibrate to room temperature (18˚C to 25˚C) before starting the assay. Thoroughly mix the reagents and samples before use by gentle agitation or swirling.
2. Do not use kit components beyond the expiration date.
3. Do not mix materials from different kit lots.
4. Do not mix strips from different plates.
5. Perform Standards, Controls and Unknowns in duplicate. Vertical alignment is recommended.
6. A standard curve should be run with each assay run or each plate run.
7. To avoid drift, the time between pipetting of the first standard and the last sample must be no longer than 30 minutes. Otherwise, results will be affected.
8. Use a clean disposable plastic pipette for each reagent, standard, control or specimen addition in order to avoid cross contamination .
9. For the dispensing of the Chromogenic Solution and Stop Solution avoid pipettes with metal parts.
10. Use a clean plastic container to prepare the Wash Solution.
11. During incubation with Chromogenic Solution, avoid direct sunlight on the microtiter plate.
12. Respect the incubation times described in the assay procedure.

B. Assay Procedure

1. **Select the required number of strips for the run.** The unused strips should be resealed in the bag with dessicant and stored at 2-8˚C.
2. Secure the strips into the holding frame.
3. **Pipette 50 µl of Solution B** into the appropriate wells for the : Standards, Controls, and Serum/plasma samples
4. **Pipette 50 µl of Solution A** into the appropriate wells for **cell culture supernatant**
5. **Pipette 100 µl of each Standard, Control, or Sample** into the appropriate wells.
6. **Pipette 50 µl of Biotin conjugate** into all the wells.
7. **Incubate** for **2 hours** at room temperature on a horizontal shaker set at 700 ± 100 rpm.
8. **Aspirate** the liquid from each well ;
9. **Wash** the plate three times by :
   a) dispensing of 0.4 ml of BioSource Wash Solution into each well ;
   b) aspirating the content of each well.
10. **Pipette 100 µl of diluted Streptavidin-HRP conjugate** into all the wells.
11. **Incubate** for **30 min.** at room temperature on a horizontal shaker set at 700 ± 100 rpm.
12. **Aspirate** the liquid from each well ;
13. **Wash** the plate four times by :
    a) dispensing of 0.4 ml of BioSource Wash Solution into each well ;
    b) aspirating the content of each well.
14. **Pipette 100 µl of Chromogenic Solution (TMB)** into all the wells.
15. **Incubate** the plate for **15 min.** at room temperature on an horizontal shaker set at 700 ± 100 rpm, avoiding direct sunlight.
16. **Pipette 100 µl of Stop Solution** into each well.
17. **Read** the absorbance within 1 hour and calculate the results as described in section XI.

XI CALCULATION OF ANALYTICAL RESULTS

A. Reading the plate with an equipment capable to record an optical density of 3.0 or more

. Read the microtiter plate at 450 nm (reference filter : 630 or 650 nm).
. Construct a standard curve by plotting the OD on the ordinate against the standard concentrations on the abscissa using either linear or semi-log graph paper and draw the curve by connecting the plotted points with straight ligne.
. Determine IL-17 concentrations of Samples or Controls.

B. Reading the plate with an equipment capable to record an optical density lower than 3

. Read the microtiter plate at 405 nm (reference filter : 630 or 650 nm).
It will result in a decrease of the OD units when compared to ODs read at 450 (as shown on the table hereafter). Nevertheless, results remain quite similar.

C. Example of a typical reference curve

The following data are for demonstration purpose only and can not be used in place of data generated at the time of assay.

IL-17 CytoScreen			Reading 450 nm (OD Units)	Reading 405 nm (OD Units)
Standard	0	pg/ml	0.026	0.020
	15.6	pg/ml	0.093	0.039
	31.3	pg/ml	0.160	0.059
	62.5	pg/ml	0.279	0.094
	125	pg/ml	0.521	0.166
	250	pg/ml	0.984	0.302
	500	pg/ml	1.934	0.576
	1000	pg/ml	3.632	1.130

XII QUALITY CONTROL

- The **two Controls** provided in the kit can be used as internal laboratory controls.
- Serum or heparin plasma pools as well as stimulated cell culture supernatants can be collected and frozen immediately in aliquot to serve as controls. Repeated freezing and thawing are not permitted.
- **Record keeping** : it is good laboratory practice to record the kit lot numbers and date of reconstitution for the reagents in use.
- **Controls** : it is recommended that Controls be routinely assayed as unknown samples to measure assay variability. It is recommended that quality controls charts be maintained to monitor the performance of the kits. Control ranges are indicated on vial labels. Out of range control results indicate the assay must be repeated. Repeat patient samples may also be used to measure interassay precision.
- **Sample handling** : strictly adhere to the instruction for handling and storage of samples. Standards, Controls, and Unknowns should be run in duplicate. A clean disposable tip should always be used to avoid carryover contamination.
- **Data reduction** : it is good practice to construct a standard curve for each run to check visually the curve fit selected by the computer program.

XIII EXPECTED RANGE (Reference Interval)

In process

XIV PERFORMANCE CHARACTERISTICS

1. **Minimum Detectable Concentration (MDC).**
The MDC is estimated to be 2 pg/ml and is defined as the IL-17 concentration corresponding to the average OD of 20 replicates of the zero standard + 2 standard deviations.

2. **Precision** (inter-assay in process)

INTRA-ASSAY

Sample	n	<X > SD (pg/ml)	CV (%)
Serum 1	19	169 ± 6	3.7
2	19	456 ± 17	3.7

INTER-ASSAY (day-to-day)

Sample	n	<X> ± SD (pg/ml)	CV (%)
Serum 1		±	
2		±	

3. **Specificity**
No significant cross-reaction was observed in presence of 300 ng of IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-16, TNF-α, IFN-γ, GM-CSF, OSM , MIP-1α, MIP-1β, LIF, MCP-1, G-CSF,GRO, IP-10, SCF, MCP-3, NAP-2 and RANTES. This IL-17 assay is specific for human natural and recombinant IL-17.

4. **Accuracy**

RECOVERY

Sample	Added IL-17 (pg/ml)	Recovered IL-17 (pg/ml)	Recovery (%)
Plasma	0	0	
	187	175	94
	375	323	86
	882	741	84
High rheumat. Factor Sample	0	0	
	187	242	129
	375	351	94
	882	702	80
Cell Cult. Med.	0	0	
	187	217	116
	375	413	110
	882	883	100

DILUTION TEST

Sample	Dilution	Theor Conc (pg/ml)	Meas. Conc. (pg/ml)
Activated Plasma	1/2	597	597
	1/4	299	256
	1/8	149	130
	1/16	75	60
	1/32	37	33
cell cult.1	1/1	450	450
	1/2	225	232
	1/4	113	120
	1/8	56	54
	1/16	28	25
cell cult.2	1/1	630	630
	1/2	315	326
	1/4	158	156
	1/8	79	85
	1/16	40	36

5. **High dose hook-effect**
A sample spiked with IL-17 up to 0.2 µg/ml gives a response higher than that obtained for the last standard point.

XV LITERATURE REFERENCES

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Immunity, 3 : 811-821.

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Human IL-17: a novel cytokine derived from T Cells
J. of Immunology, : 5483-5486.

4. FOSSIEZ F., et al (1996)
T Cell IL-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines.
J. of Exp.Med.183(6):2411-2415

XVI SUMMARY OF ASSAY PROCEDURE

	Standards (µl)	Serum/ plasma samples (µl)	Culture Supernatant urine (µl)
Solution B	50	50	-
Solution A	-	-	50
Standards (0-5), Controls	100	-	-
Serum/plasma samples	-	100	-
Culture supernatant/urines	-	-	100
Biotin-conjugate	50	50	50
Incubate for 2 hours at R.T. with continuous shaking (700 RPM) **Aspirate** the contents of each well **Wash** 3 times with 0.4 ml of Wash Solution and aspirate			
Streptavidin-HRP	100	100	100
Incubate for 30 min. at R.T. with continuous shaking (700 RPM) **Aspirate** the contents of each well **Wash** 4 times with 0.4 ml of Wash Solution and aspirate			
Chromogenic Solution	100	100	100
Incubate 15 min. at R.T. with continuous shaking			
Stop Solution	100	100	100
Read on a microtiter plate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm).			

BioSource Catalogue Nr : KAC1591 / KAC1592	P.I. Number : 1700568	Date of issue : 20 March 2000

Before use, read this Package Insert.

IL-17 Cytoscreen

For research use only. Not for use in diagnostic procedures.

An immunoenzymometric assay for the quantitative measurement of human Interleukin-17 in serum, plasma, cell culture medium or other biological fluids.

I GENERAL INFORMATION

A. Proprietary Name : BIOSOURCE IL-17 Cytoscreen kit

B. Catalogue Number : KAC1591 : 96 determinations
KAC1592 : 2 x 96 determinations

C. Manufactured by : BioSource Europe S.A.
Rue de l'Industrie, 8 B-1400 Nivelles Belgium.

For technical assistance or ordering information contact :
Telephone numbers : (Voice) +32/67/88.99.00 (Fax) +32/67/88.99.96

II APPLICATION AND INTENDED USE

Interleukin-17, also called CTLA-8, is a glycoprotein of 155 amino acids .
IL-17 is secreted by activated CD4+ T Cells as a mixture of homodimeric glycosylated and non-glycosylated polypeptides.While devoid of direct effects on cells of haematopoietic origin, hIL-17 and the product of its viral counterpart, ORF13, stimulate epithelial, endothelial and fibroblastic cells to secrete cytokines such as Interleukin-6 (IL-6), IL-8, granulocyte-colony stimulating factor (G-CSF) and prostaglandin E2 (PGE2).
IL-17 may constitute an early initiator of the T Cell dependent inflammatory reaction and an essential element of the cytokine network that bridges the immune system to hematopoiesis.
IL-17 also plays a limited proinflammatory role in Tcell driven inflammatory pathological processes such as psoriasis or sarcoidosis.