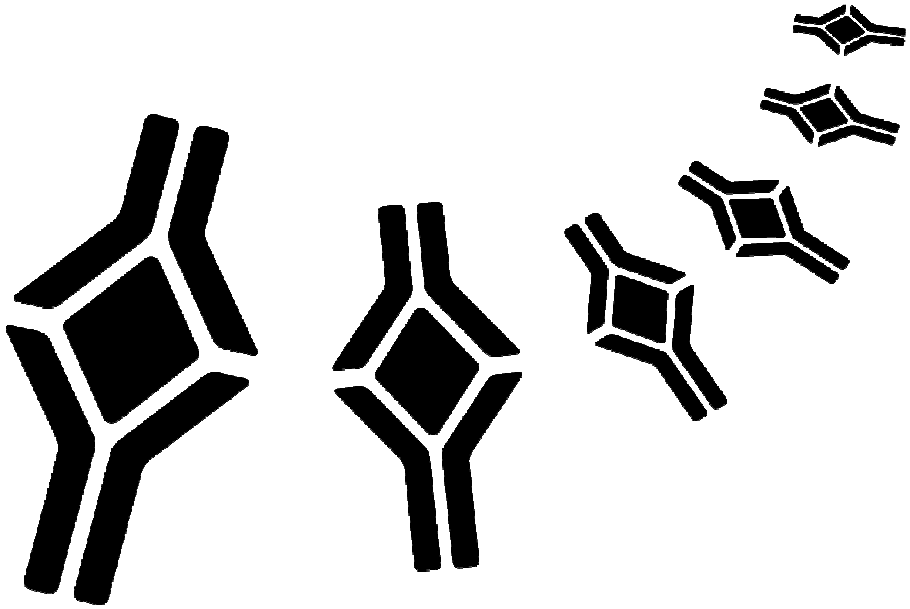


HUMAN INTERLEUKIN-10 ELISA, HIGH SENSITIVITY

Product Data Sheet

Cat. No.: RGP015R

For Research Use Only

VERSION 51 180612 46

CONTENTS

This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The Human Interleukin-10 ELISA, High Sensitivity is to be used for research use only quantitative determination interleukin-10 (IL-10) in human sera, plasmas, buffered solutions or cell culture media. The assay will recognize both natural and recombinant human IL-10. **This kit is intended for research use only and is not to be used in diagnostic procedures.**

2. PRINCIPLE OF THE METHOD

The IL-10 Kit is a solid phase sandwich Enzyme Linked-Immuno- Sorbent Assay (ELISA). A monoclonal antibody specific for IL-10 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-10 concentrations and unknowns are pipetted into these wells.
During the first incubation, the IL-10 antigen and a biotinylated monoclonal antibody specific for IL-10 are simultaneously incubated. After washing, the enzyme (streptavidin-peroxydase) is added. All the unbound enzyme is removed by washing and the first amplification step is performed by adding the Biotine-Tyramine reagent. Under the action of HRP, a biotine polymerisation reaction occurs in the region of the HRP linked to the detection antibody. After washing the second amplification step is performed and the polymerised biotine is revealed by a new streptavidin-HRP step. Finally after washing, the substrate is added. The intensity of this coloured product is directly proportional to the concentration of IL-10 present in the samples.

3. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	**COLOUR CODE**	**Quantity**	**State**
Antibody Coated Microtiter Strips		96 wells	Ready to use
Plastic cover		2	
Standard: 50 pg/ml	Yellow	2 vials	Reconstitute with the volume of standard diluent indicated on the Quality Control Sheet. (See Reagents Preparation).
Standard Diluent buffer	Black	1 vial (25 ml)	10X concentrate. Dilute in distilled Water.
Standard Diluent: human serum	Black	1 vial (7 ml)	Ready to use
Biotinylated anti-IL-10	Red	1 vial (0.4 ml)	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	Red	1 vial (7.5 ml)	Ready to use
Streptavidin-HRP		2 vials (5 µl)	0.5 ml of HRP-Diluent before further dilutions
Amplification Diluent	Brown & blue spot	1 vial (25 ml)	Ready to use
Amplifier*	Yellow	1 vial (200 µl)	Dilute in Amplification buffer.
HRP Diluent	Red	1 vial (25 ml)	Ready to use
Washing Buffer	White	1 vial (10 ml)	200X concentrate. Dilute in distilled Water
Chromogen TMB :		1 vial (11 ml)	Ready to use
H_2SO_4 : Stop Reagent	Black	1 vial (11 ml)	Ready to use

*Reagent contains ethyl alcohol.

4. MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water.
- Pipettes : 10 µl, 50 µl, 100 µl, 200 µl and 1000 µl.
- Vortex mixer and magnetic stirrer.
- Rotator set

5. SAFETY

- For research use only.
- The 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/NIH Health manual: "Biosafety in Microbiological and Biomedical Laboratories 4th Edition" 1999.
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette with mouth.

6. PROCEDURAL NOTES/LAB. QUALITY CONTROL

1. When not in use, kit components should be stored refrigerated as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards and controls should be discarded directly after resuspension and use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different batches.
5. Do not use reagents beyond the expiration date of the kit.
6. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.
11. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
12. Respect incubation times described in the assay procedure.

7. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants - Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.
Serum – Avoid any inintentional stimulation of the cells by the procedure. Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. For that, after clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.
Plasma - EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.
Storage - If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, they should be removed prior to assay by centrifugation or filtration.
Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before assaying.

8. PREPARATION OF REAGENTS

8.1 Standard buffer diluent10X concentrate

Dilute 10 times with distilled water before use.

8.2 Standards

Depending on the type of samples you are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. You should reconstitute standard vials with the most appropriate standard diluent. For serum and plasma samples use standard diluent human serum and for cells culture supernatants use Standard diluent buffer. Standard vials have to be reconstituted with the volume of appropriate standard diluent indicated in the Quality Control Sheet. This reconstitution gives a stock solution of 50 pg/ml IL-10. Allow standard to stand for 5 minutes with gentle swirling prior to making dilutions. Serial dilutions of standard must be made before each assay and cannot be stored.

8.3 Samples

Normal sera and plasmas may be applied undiluted. Nevertheless, sera or plasmas from patients with various pathologies may be applied undiluted and diluted (to prevent too high concentrations). As IL-10 concentrations may vary considerably in cell supernatant samples, it is not easy to recommend a dilution factor. For example, unknown cell supernatant samples may also be tested undiluted and diluted.

8.4 Dilution of biotinylated anti-IL-10

Dilute the biotinylated anti-IL-10 with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
32	80	2120
48	120	3180
96	240	6360

8.5 Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 µl vial of Streptavidin-HRP . DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS.

From the previous solution two others dilutions must be prepared: one for the step “J” and one for the step “P“.

Extemporaneous preparations are recommended. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial: see hereafter the table for volumes to pipette.

	Preparation of Streptavidin solution 1 – Step J		Preparation of Streptavidin solution 2 – Step P	
Number of Wells	**Streptavidin-HRP(µl)**	**Strep-HRP Diluent (ml)**	**Streptavidin-HRP (µl)**	**Strep-HRP Diluent (ml)**
16	10	1,990	32	1,900
32	20	3,980	64	3,800
48	30	5,970	96	5,700
96	60	11,940	192	11,400

8.6 Dilution of Amplifier

Extemporaneous preparations are recommended. Following the number of wells to be used, further dilutions of amplifier should be made with amplification diluent in a clean glass vial: see hereafter the table for volumes to pipette.

Number of Wells	Amplifier (µl)	Amplification Diluent (ml)
16	20	1,980
32	40	3,960
48	60	5,940
96	120	11,880

8.6 Washing Buffer 200X concentrate

Dilute 200 times in distilled water.

9. ASSAY METHOD

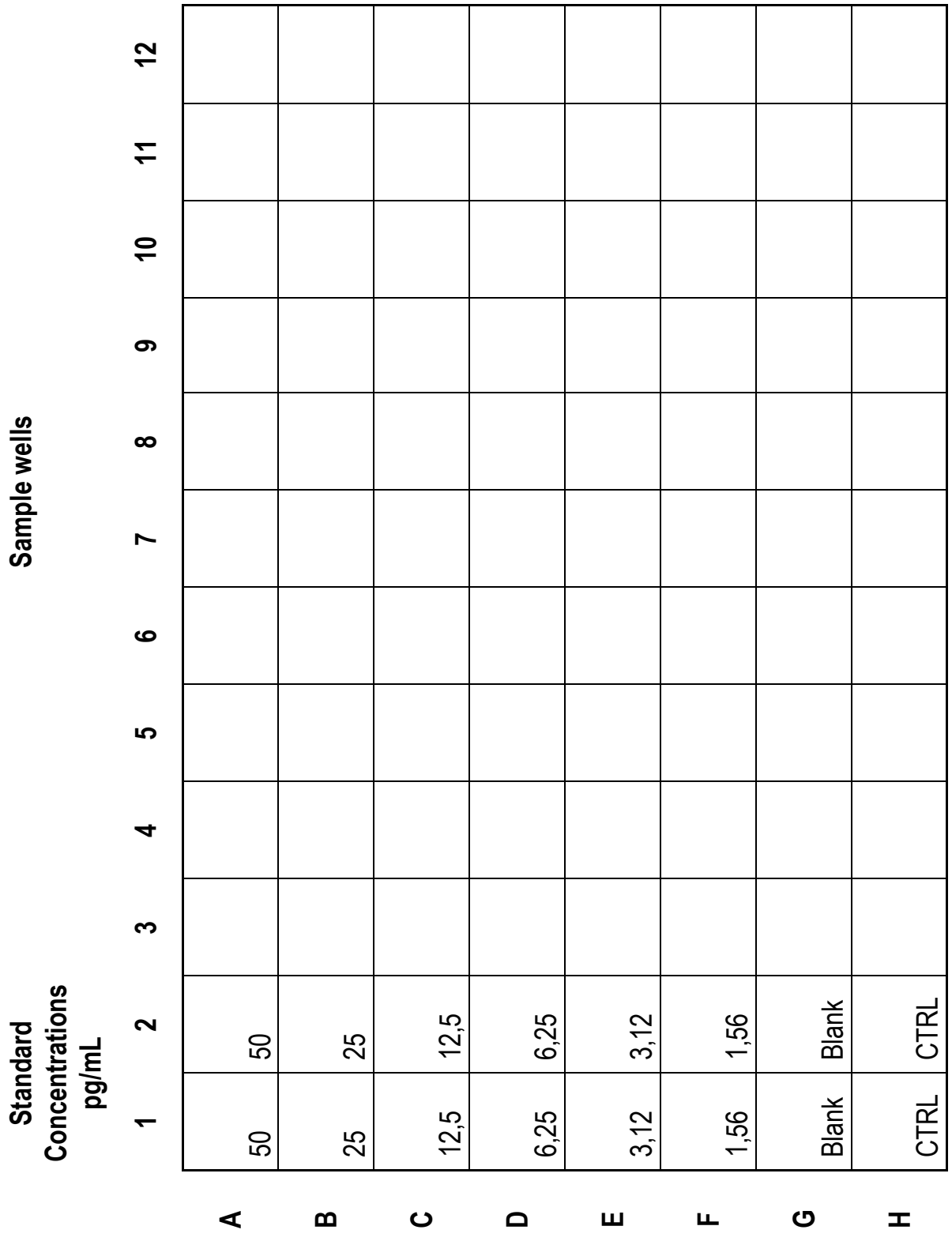
a) Before use, mix all reagents thoroughly without making foam.
b) Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank should be assayed **in duplicate**. Remove sufficient microwell strips from the pouch.
c) Add 100 µl of appropriate standard diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-10 standard dilutions ranging from 50 to 1.56 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2). Alternatively these dilutions can be made in separate tubes and diluted standard pipetted directly into wells.
d) Add 100 µl of appropriate standard diluent to the blank wells (G1-G2).
e) Add 100 µl of sample to sample wells
f) Preparation of biotinylated anti-IL-10: see reagents preparation.
g) Add 50 µl of diluted biotinylated anti-IL-10 to all wells.
h) Cover with a plate cover and incubate for 2 hours at room temperature (18°C - 22°C) with slow shaking
i) Remove the cover and wash the plate as follows:
    1) aspirate the liquid from each well ;
    2) dispense 0.3 ml of washing solution into each well ;
    3) aspirate again the content of each well ;
    4) Repeat steps 2) and 3) two times.
j) Prepare streptavidin-HRP solution 1 just before use: see reagents preparation.
k) Distribute 100 µl of the streptavidin-HRP solution 1 to all wells, including blank wells.
l) Cover and incubate 20 min at room temperature with slow shaking.
m) Remove the cover and wash according to step i)
n) Add 100 µl of amplifier dilution prepared according the instructions for the preparation of reagents and incubate 15 minutes with slow shaking.
o) Remove the cover and wash according to step i).
p) Prepare streptavidin-HRP solution 2 just before use: see reagents preparation.
q) Distribute 100 µl of the streptavidin-HRP solution 2 to all wells, including blank wells.
r) Cover and incubate 20 min at room temperature with slow shaking.
s) Remove the cover and wash according to step i).
t) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for around 5 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

u) Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. **The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable.**
v) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of stop reagent (1N sulphuric acid) into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read rapidly after the addition of stop reagent.
w) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

Note: In case of incubation without shaking the O.D values may be lower than with shaking; in this case let the color develop longer in order to obtain correct OD values.

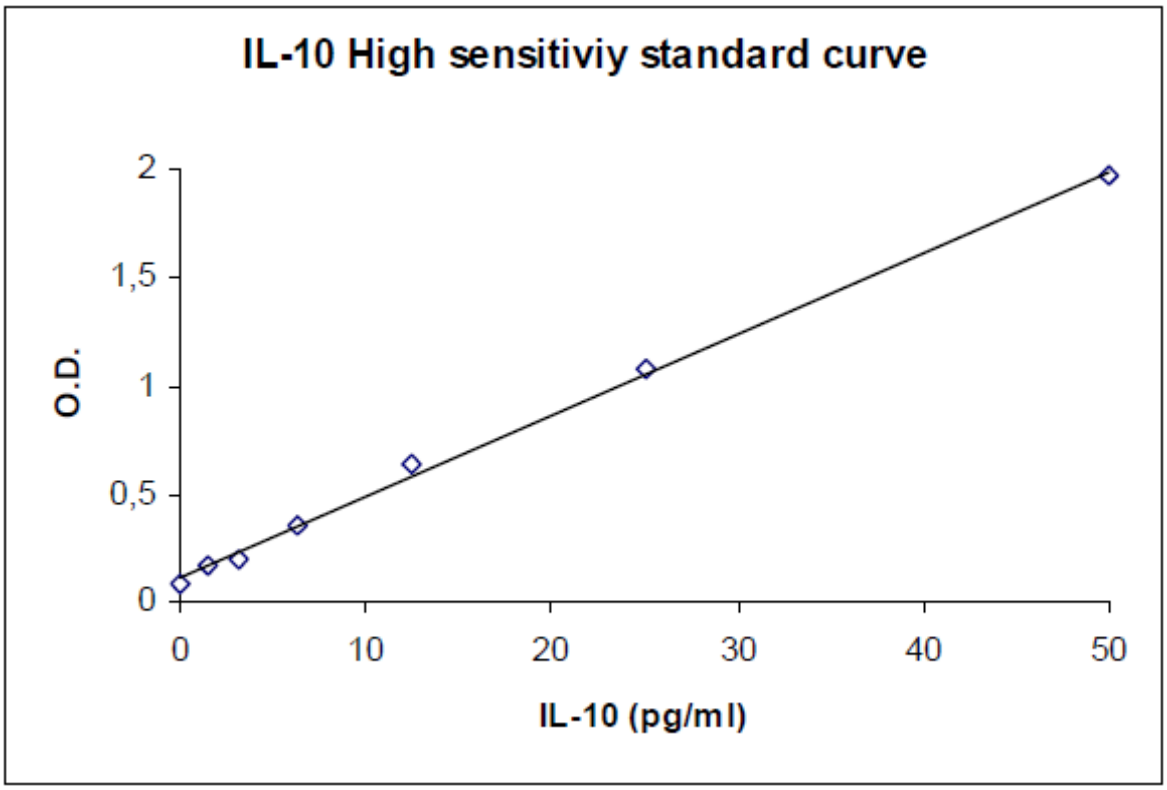
All incubation steps except substrate TMB and acid are performed under slow shaking at room temperature.

10. SUGGESTED PLATE SCHEME



11. DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding IL-10 standard concentration on the horizontal axis. The amount of IL-10 in each sample is determined by extrapolating OD values to IL-10 concentrations using the standard curve.



Typical IL-10 High sensitivity standard curve ranging from 1.56 to 50 pg/mL

12. LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 50 pg/ml standard curve point. The dose-response is non-linear in this region and therefore it is difficult to obtain accurate concentration. Concentrated samples (> 50 pg/ml) have to be diluted with standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been investigated. The rate of degradation of native IL-10 in various matrices has not been investigated.

13. PERFORMANCES AND CHARACTERISTICS

13.1 Sensitivity
This has been determined by adding 3 standard deviations to the mean concentration of 40 zeros. The minimum detectable dose of IL-10 was less than 1.30 pg/ml.

13.2 Specificity
Ten specificities were tested with concentrations higher than IL-10 curve concentrations. No cross reaction was observed for concentrations ranging from 250 to 15.62 pg/ml for IL-1 α and β, IL-2, IL-5, IL-6, IL-8, IL-12p40, FasL, TNF-α and IFN-γ.

13.3 Spiking - Recovery
The spiking recovery was evaluated by spiking three levels of IL-10 into four different pooled human sera and one cell culture medium. Recovery was evaluated with one test. The recovery in pooled human sera ranged from 59 to 93% with an average of 80%. In cell culture medium, recovery was 94%. Note that recovery in plasma appeared lower than in sera or culture medium (data not shown).

13.4 Precision
Four pooled human serum samples, one cell culture medium and one pooled plasma sample with various concentrations of IL-10 were tested for repeatability and reproductibility. Each assay was carried out with 3 duplicates of each sample. Three independent assays were performed. The intra-assay and inter-assay coefficient of variation has been calculated to be 7.8% and 10.2% respectively.

13.5 Linearity of Dilution

Two pooled human sera, one pooled human plasma and one cell culture medium samples containing different concentrations of IL-10 were serially diluted in standard buffer diluent. Linearity was evaluated on 4 dilutions. The linear regression of samples versus the expected concentration yielded a quote slope of 0.993.

13.6 Expected values

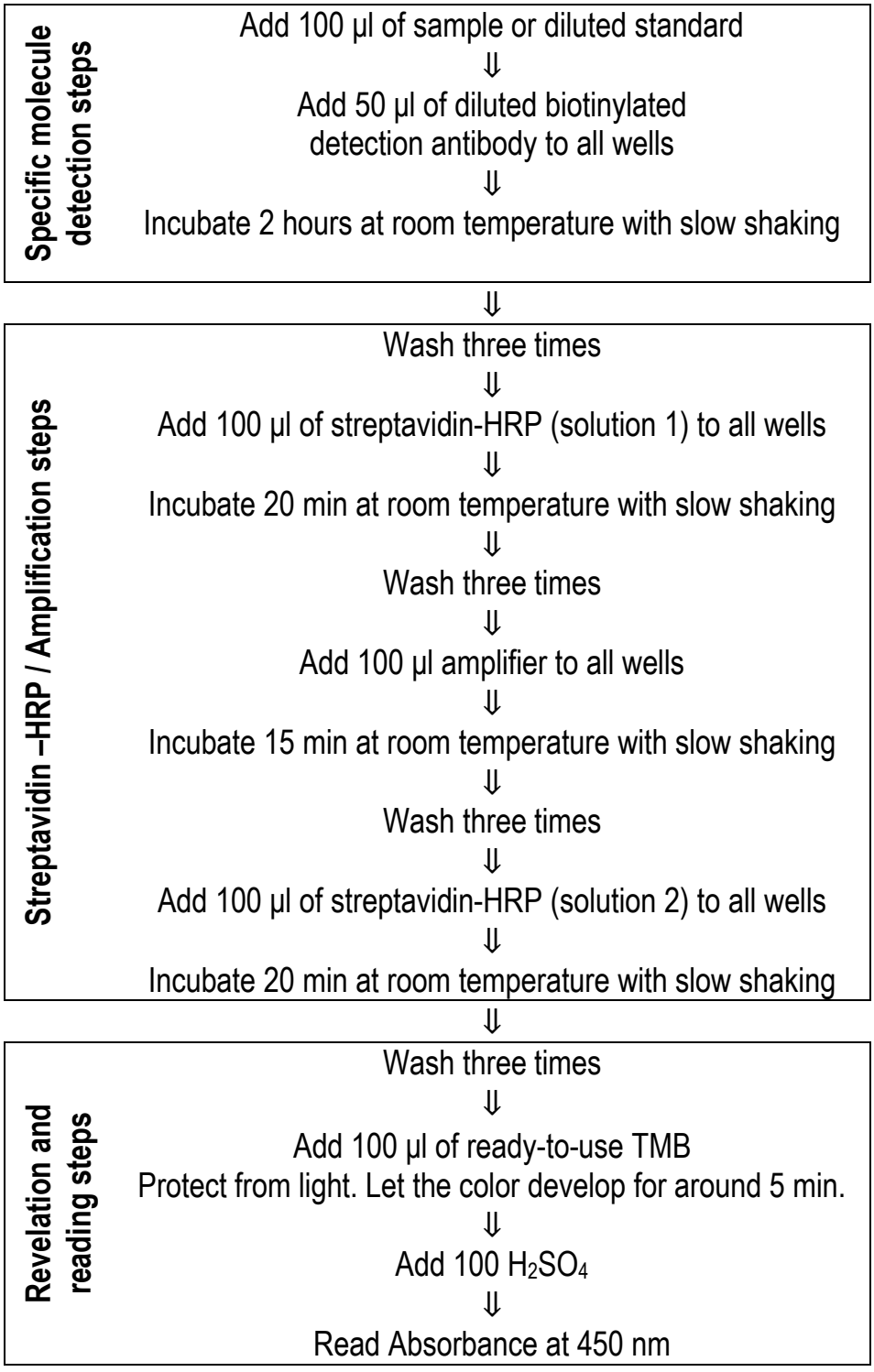
16 sera from healthy individual donors were tested undiluted in duplicates. 15 sera were negative and one was slightly positive: 4.12 pg/ml.

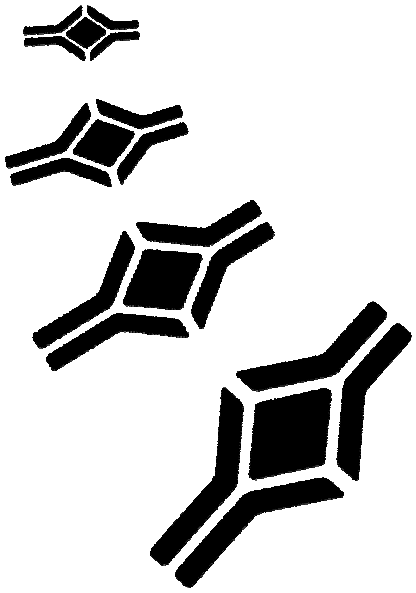
Study: _____ **Specifity:** _____
Date: _____ **Plate n°:** _____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

14. ASSAY PROCEDURE SUMMARY

Total procedure length 3 h 00 mn





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