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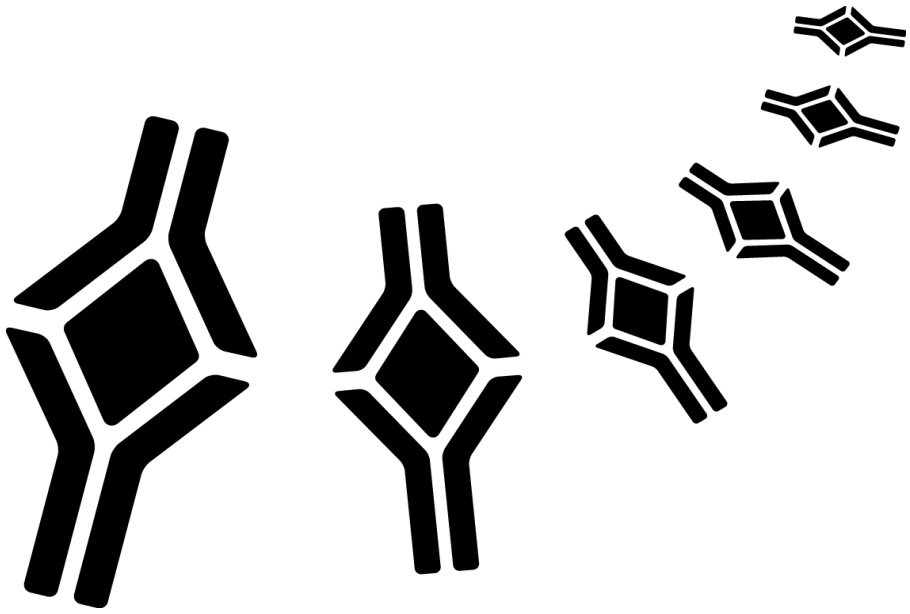
Research
and Diagnostic Products



# HUMAN INTERLEUKIN-10 ELISA, HIGH SENSITIVITY

**Product Data Sheet**

**Cat. No.: RGP015R**

**For Research Use Only**

## 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.**

# 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

- Microtitre plate reader fitted with appropriate filters (450 nm required with optional 630 nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single channel micropipettes with disposable tips
- 50-300 µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Orbital shaker
- Miscellaneous laboratory plastic and/or glass, if possible sterile

## 5. STORAGE INSTRUCTIONS

Store kit reagents between 2 and 8°C. Immediately a fter use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

**Wash Buffer:** Once prepared store at 2-8°C for up to 1 week
**Standard Diluent Buffer :** Once prepared store at 2-8°C for up to 1 week
**Standards:** Once prepared use immediately and do not store
**Biotinylated Secondary Antibody:** Once prepared use immediately and do not store
**Streptavidin-HRP:** Once prepared use immediately and do not store

## 6. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants, serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation
**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.
**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 use. 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.

## 7. SAFETY& PRECAUTIONS FOR USE

- 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" 1984
- The human serum included in this kit have been tested and found non reactive for HbsAg, anti HIV1 & 2 and anti VHC. 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
- Laboratory gloves should be worn at all times
- Avoid any skin contact with $H_2SO_4$ and TMB. In case of contact, wash thoroughly with water
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- 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
- Use a clean plastic container to prepare the washing solution
- Thoroughly mix the reagents and samples before use by agitation or swirling
- 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

- 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 off properly
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance's within 1 hour after completion of the assay
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells
- Follow incubation times described in the assay procedure
- Dispense the TMB solution within 15 min of the washing of the microtitre plate

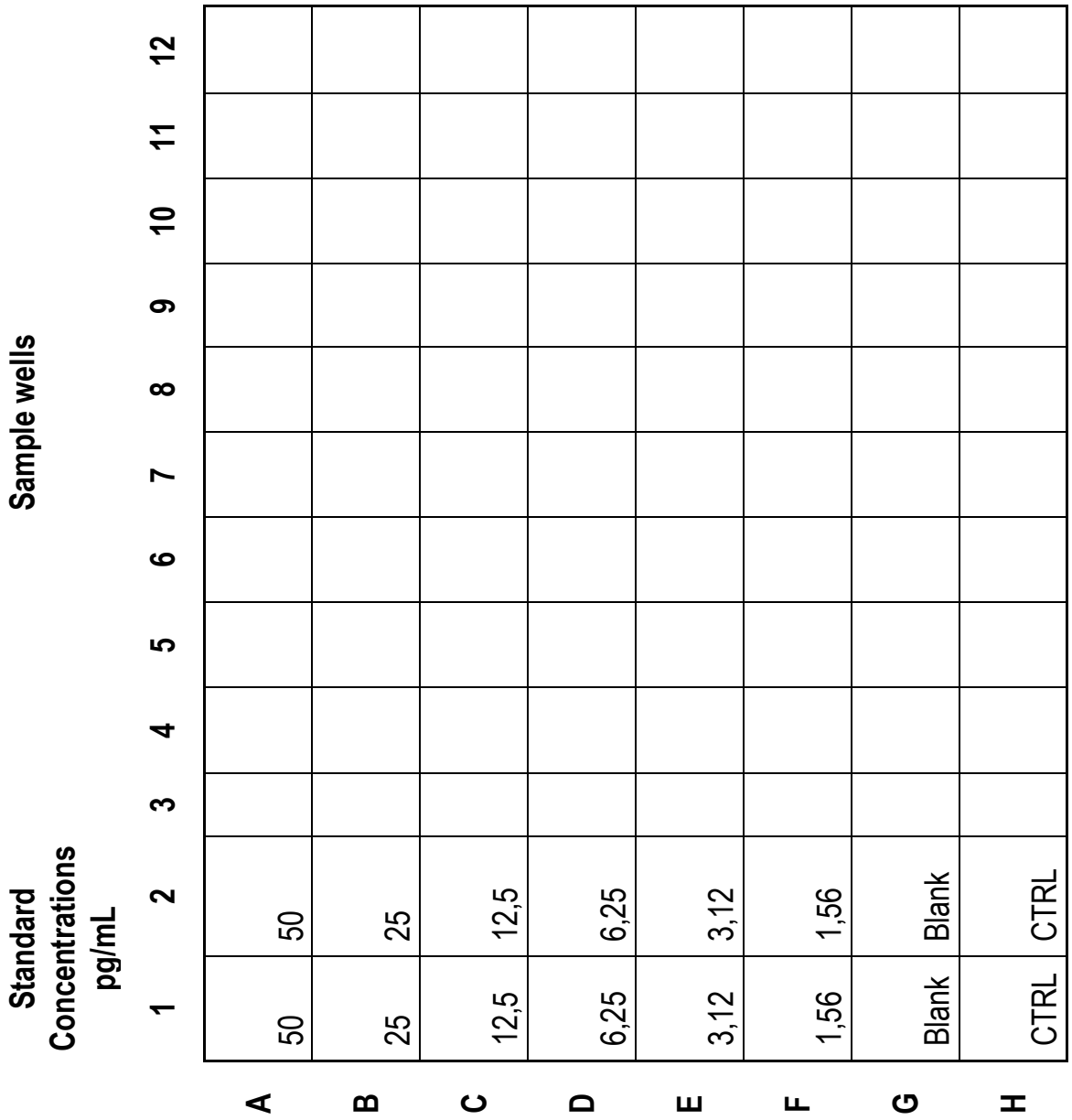
# 8. PREPARATION OF REAGENTS

**Bring all reagents to room temperature before use**

## 8.1 Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard, zero and control should be tested in duplicate. Remove sufficient Microwell Strips for testing from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

**Example plate layout (example shown for a 6 point standard curve)**




*All remaining empty wells can be used to test samples in duplicate*

## 8.2. Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2°-25°C.

## 8.3. Preparation of Standard Diluent Buffer

Add the contents of the vial (10x concentrate) to 225 ml of distilled water before use.
This solution can be stored at 2-8°C for up to 1 week

## 8.4 Preparation of Standard

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 must be reconstituted with the volume of standard diluent indicated in the Quality Control Sheet. This reconstitution gives a stock solution of 50 pg/ml of IL-10. **Mix the reconstituted standard gently by inversion only.** Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 50 to 1.56 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200 µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 50 pg/ml
- Add 100 µl of appropriate standard diluent to the remaining standard wells B1 and B2 to F1 and F2
- Transfer 100 µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Continue this 1:1 dilution using 100 µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 50 pg/ml to 1.56 pg/ml
- Discard 100 µl from the final wells of the standard curve (F1 and F2)

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

## 8.5 Preparation of 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.

## 8.6 Preparation of Biotinylated anti-IL-10

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-IL-10 with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

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

## 8.7 Preparation of Streptavidin-HRP solutions 1 and 2

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.
Dilute the 5 µl vial with 0.5 ml of HRP diluent immediately before use**.** This pre-dilution will be used for Step 5 and step 11. Do-not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

| | **Preparation of Streptavidin solution 1 – Step 5** | | **Preparation of Streptavidin solution 2 – Step 11** | |
|---|---|---|---|---|
| **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 |

## 8.8 Dilution of Amplifier

Extemporaneous preparations are recommended. Dilute the Amplifier with the Amplification diluent in an appopriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

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

# 9. METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.
Prepare all reagents as shown in section 8.
Note: final preparation of Biotinylated anti-IL-10 (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

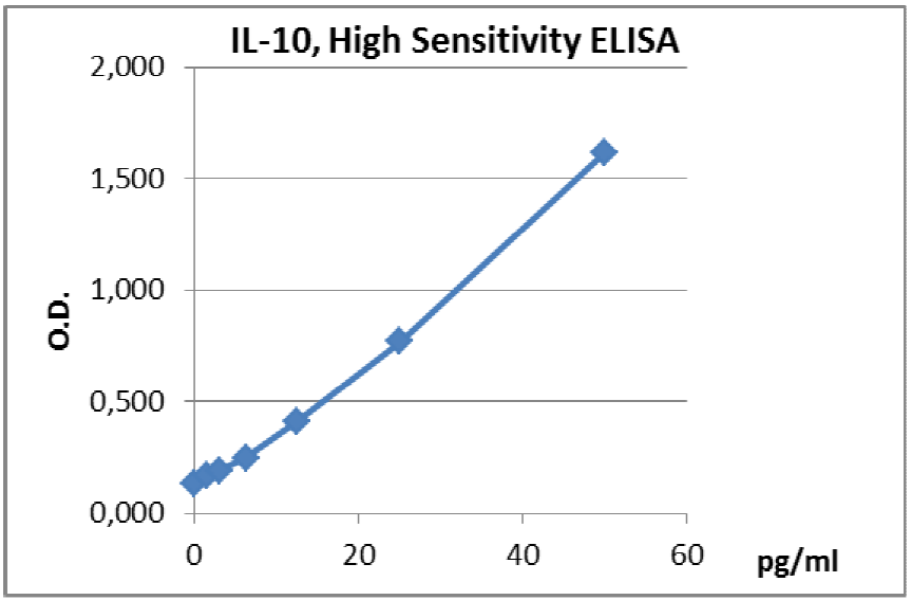
| **Assay Step** | | **Details** |
|---|---|---|
| **1.** | **Addition** | Add 100 µl of each **Standard, Sample and zero (appropriate standard diluent buffer)** in duplicate to appropriate number of wells |
| **2.** | **Addition** | Add 50 µl of diluted **biotinylated anti-IL-10** to all wells |
| **3.** | **Incubation** | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **2 hour(s)** |
| **4.** | **Wash** | Remove the cover and wash the plate as follows:<br>a) Aspirate the liquid from each well<br>b) Dispense 0.3 ml of **1x washing solution** into each well<br>c) Aspirate the contents of each well<br>d) Repeat step b and c another two times |
| **5.** | **Addition** | Add 100 µl of **Streptavidin-HRP solution 1** into all wells |
| **6.** | **Incubation** | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **20 min** |
| **7.** | **Wash** | Repeat wash step 4. |
| **8.** | **Addition** | Add 100 µl of **Amplifier** to all wells |
| **9.** | **Incubation** | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **15 min** |
| **10.** | **Wash** | Repeat wash step 4. |
| **11.** | **Addition** | Add 100 µl of **Streptavidin-HRP solution 2** into all wells |
| **12.** | **Incubation** | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **20 min** |
| **13.** | **Wash** | Repeat wash step 4. |
| **14.** | **Addition** | Add 100 µl of ready-to-use **TMB Substrate Solution** into all wells |
| **15.** | **Incubation** | Incubate in the dark for **10-20 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil. |
| **11.** | **Addition** | Add 100 µl of **$H_2SO_4$:Stop Reagent** into all wells |
| **Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).** | | |

*****_Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range_

**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.

## 10. DATA ANALYSIS

Calculate the average absourbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.
Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding Human 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.




**Example IL-10 Standard curve**

| Standard | IL-10 Conc pg/ml | OD (450 nm) mean | CV (%) |
|---|---|---|---|
| 1 | 50 | 1,616 | 4,7 |
| 2 | 25 | 0,770 | 2,5 |
| 3 | 12,5 | 0,412 | 2,1 |
| 4 | 6,25 | 0,247 | 0,3 |
| 5 | 3,12 | 0,191 | 0,4 |
| 6 | 1,56 | 0,165 | 4,3 |
| zero | 0 | 0,129 | - |

**Note**: curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

## 11. LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration. The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use. As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

## 12. PERFORMANCES AND CHARACTERISTICS

### 12.1 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.

### 12.2 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.

### 12.3 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).

### 12.4 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.

## 12.5 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.

## 12.6 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.

# 13. ASSAY PROCEDURE SUMMARY

**Total procedure length 3 h 00 mn**

**Specific molecule detection steps**

Add 100 µl of sample or diluted standard
⇓
Add 50 µl of diluted biotinylated detection antibody to all wells
⇓
Incubate 2 hours at room temperature with slow shaking

⇓

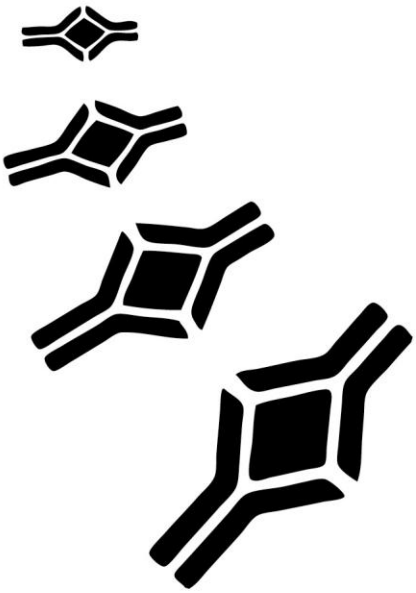
**Streptavidin –HRP / Amplification steps**

Wash three times
⇓
Add 100 µl of streptavidin-HRP (solution 1) to all wells
⇓
Incubate 20 min at room temperature with slow shaking
⇓
Wash three times
⇓
Add 100 µl amplifier to all wells
⇓
Incubate 15 min at room temperature with slow shaking
⇓
Wash three times
⇓
Add 100 µl of streptavidin-HRP (solution 2) to all wells
⇓
Incubate 20 min at room temperature with slow shaking

⇓

**Revelation and reading steps**

Wash three times
⇓
Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for around 10-20 min.
⇓
Add 100 $H_2SO_4$
⇓
Read Absorbance at 450 nm



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