cellsciences.com

Human IL-10 High Sensitivity ELISA KIT

Catalog No: CDK037A
CDK037B

Quantity: 1 x 96 tests
2 x 96 tests

SPECIFICITY :	Recognizes both natural and recombinant human IL-10
RANGE :	1.56 pg / ml - 50 pg / ml
SENSITIVITY :	< 1.30 pg / ml
INCUBATION :	3 hours
SAMPLE TYPES :	Serum Plasma Cell culture supernatant
SAMPLE SIZE :	100 µl
CROSS REACTION :	No cross reactivity with other human cytokines
KIT CONTENT :	Pre-coated 12 strip plates, Biotinylated Secondary Antibody, Amplifier, Standard, Buffers, Streptavidin-HRP, TMB, Stop Reagent

1. INTENDED USE

The Human high sensitivity IL-10 ELISA is to be used for the *in-vitro* 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 has been configured for research 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. The entire unbound enzyme is removed by washing and the first amplification step is performed by adding the **Biotin-Tyramine reagent**. Under the action of HRP, a **biotin polymerization reaction** occurs in the region of the HRP linked to the detection antibody. After washing the second amplification step is performed and the polymerized biotin is revealed by a new streptavidin-HRP step. Finally after washing, the substrate is added. The intensity of this colored product is directly proportional to the concentration of IL-10 present in the samples.

Cell Sciences, Inc.
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888-769-1246
Phone: 978-572-1070
Fax: 978-992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

3. REAGENTS PROVIDED AND RECONSTITION

Reagents (Store@2-8°C)	Quantity 1 x 96 well kit	Reconstitution
P: 96 well microtiter strip plate	1	Ready to use (Pre-coated).
A: Plastic plate covers	2	n/a
B: Standard: 50 pg/ml	3	Reconstitute as directed on the vial (see Assay preparation, section 8).
C: Standard Diluent (Buffer)	1 (25 ml)	10x Concentrate, dilute in distilled water (see reagent preparation, section 8).
D: Standard Diluent: Human Serum	1 (7 ml)	Ready to use.
E: Biotinylated anti-IL-10	1 (0.4 ml)	Dilute in Biotinylated Antibody Diluent (see Assay preparation, section 8).
F: Biotinylated Antibody Diluent	1 (7.5 ml)	Ready to use.
G: Streptavidin-HRP	2 (5 µl)	Add 0.5 ml of HRP diluent prior to use (see Assay preparation, section 8).
H: Amplification Diluent	1 (25 ml)	Ready to use.
I: Amplifier*	1 (200 µl)	Dilute in Amplification Buffer.
J: HRP Diluent	1 (25 ml)	Ready to use.
K: Wash Buffer	1 (10 ml)	200x Concentrate dilute in distilled water (see Assay preparation, section 8).
L: TMB Substrate	1 (24 ml)	Ready to use.
M: H_2SO_4 stop reagent	1 (11 ml)	Ready to use.

*Reagent contains ethyl alcohol

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter 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 after use remaining reagents should be returned to cold storage (2-8°C). The expiration date of the kit and reagents is stated on box front labels. Expiration 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 unintentional 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. 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 repeated freeze-thaw cycles and stored frozen at -80°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 haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

7. SAFETY AND 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 has 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 color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances 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 immediately after washing the microtiter plate.

8. ASSAY PREPARATION

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 and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the 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)

	Standards (pg/mL)		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	50	50										
B	25	25										
C	12.5	12.5										
D	6.25	6.25										
E	3.12	3.12										
F	1.56	1.56										
G	zero	zero										
H												

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˚-8˚C for up to 1 week.

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 biological 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 shown on the vial immediately prior to use. 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 to1.56 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 required	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:

	Streptavidin-HRP solution 1 (Step 5)		Streptavidin-HRP solution 2 (Step 11)	
Number of wells required	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (ml)	Streptavidin-HRP (µl)	Streptavidin-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. Preparation of Amplifier

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

Number of wells required	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.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **1 hour.**
3.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.3 ml of **1x washing solution** into each well. c) Aspirate the contents of each well. d) Repeat step b and c another two times.
4.	Addition	Add 50 µl of diluted **biotinylated anti-IL-10** to all wells.
5.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **1 hour.**
6.	Wash	Repeat wash step 3.
7.	Addition	Add 100 µl of **Streptavidin-HRP solution 1** into all wells.
8.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **20 min.**
9.	Wash	Repeat wash step 3.
10.	Addition	Add 100 µl of diluted **Amplifier** to all wells.
11.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **15 min.**
12.	Wash	Repeat wash step 3.
13.	Addition	Add 100 µl of **Streptavidin-HRP solution 2** into all wells.
14.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) with slow shaking for **20 min.**

15.	Wash	Repeat wash step 3.
16.	Addition	Add 100 µl of ready-to-use **TMB Substrate Solution** into all wells.
17.	Incubation	Incubate in the dark for **10-20 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
18.	Addition	Add 100 µl of **H_2SO_4:Stop Reagent** into all wells.
Read the absorbance value of each well (immediately after step 18) 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 color 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.

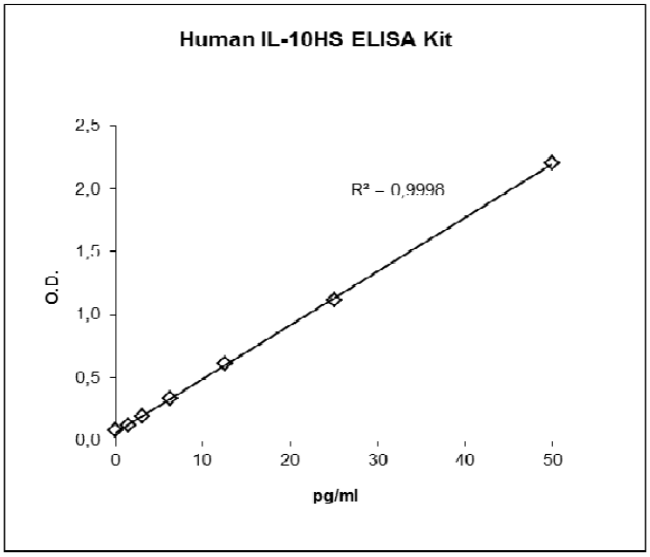
DATA ANALYSIS

Calculate the average absorbance 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 against IL-10 standard concentrations using the standard curve**.**

Example of IL-10 Standard curve

Standard	IL-10 Conc pg/ml	OD (450nm) mean	CV (%)
1	50	2,206	1,6
2	25	1,118	4,5
3	12.5	0,611	0,9
4	6.25	0,337	6,7
5	3.12	0,193	2,9
6	1.56	0,122	0,6
Zero	0	0,083	-



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.

10. Assay limitations

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.**

11. Performance Characteristics

Sensitivity

This has been determined by adding 3 standard deviations to the mean concentration of 38 zero samples. The minimum detectable dose of IL-10 was less than **0.98 pg/ml**.

Specificity

Ten specificities were tested with concentrations higher than IL-10 curve concentrations. No cross reaction was observed for concentrations for IL-1α and β, IL-2, IL-5, IL-6, IL-8, IL-12, TNF-α, IFN-γ and mIL-10.

Precision

Intra-Assay
Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in culture media and 2 in standard diluent with samples containing different concentrations of IL-10. 2 standard curves were run on each plate. **The overall intra-assay coefficient of variation was calculated to be 4.3%.**

Session	*Sample*	Mean IL-10 pg/ml	SD	CV
Session 1	*Sample 1*	22.78	0.62	**2.73**
	Sample 2	9.31	0.21	**2.29**
	Sample 3	22.76	1.51	**6.61**
	Sample 4	9.27	1.05	**11.36**
	Sample 5	20.84	0.26	**1.24**
	Sample 6	8.47	0.21	**2.44**
Session 2	*Sample 1*	26.21	0,68	**2.60**
	Sample 2	10.81	0.50	**4.61**
	Sample 3	24.64	1.49	**6.06**
	Sample 4	8.67	0.54	**6.25**
	Sample 5	24.46	1.03	**4.22**
	Sample 6	9.22	0.39	**4.21**
Session 3	*Sample 1*	21.5	1.39	**6.45**
	Sample 2	8.18	0.17	**2.06**
	Sample 3	19.31	0.27	**1.38**
	Sample 4	8.19	0.17	**2.09**
	Sample 5	20.27	1.29	**6.34**
	Sample 6	8.04	0.28	**3.44**

Inter-Assay
Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two technicians. Each assay was carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in culture media and 2 in standard diluent with samples containing different concentrations of IL-10. Two standard curves were run on each plate. **The calculated overall coefficient of variation was 6.3%.**

	Sample 1	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*	*Sample 6*
Mean IL-10 pg/ml	24	9	24	9	23	9
SD	1	1	3	0	2	0
CV	4.0	8.5	10.9	3.8	6.6	3.8

Dilution Parallelism

Two spiked human serum with different levels of IL-10 were analyzed at three serial two fold dilutions (1:2-1:16) with two replicates each. Recoveries ranged from 90% to 117% with an overall mean recovery of 101%.

Spike Recovery

The spike recovery was evaluated by spiking two concentrations of recombinant IL-10 in human serum and culture media in three experiments. Recoveries ranged from 70% to 101% with an overall **mean recovery of 85%.**

Expected Values

A panel of 20 sera and 20 plasmas coming from apparently healthy blood donors was tested for IL-10. All were below the detection level <0.98pg/ml.

Stability

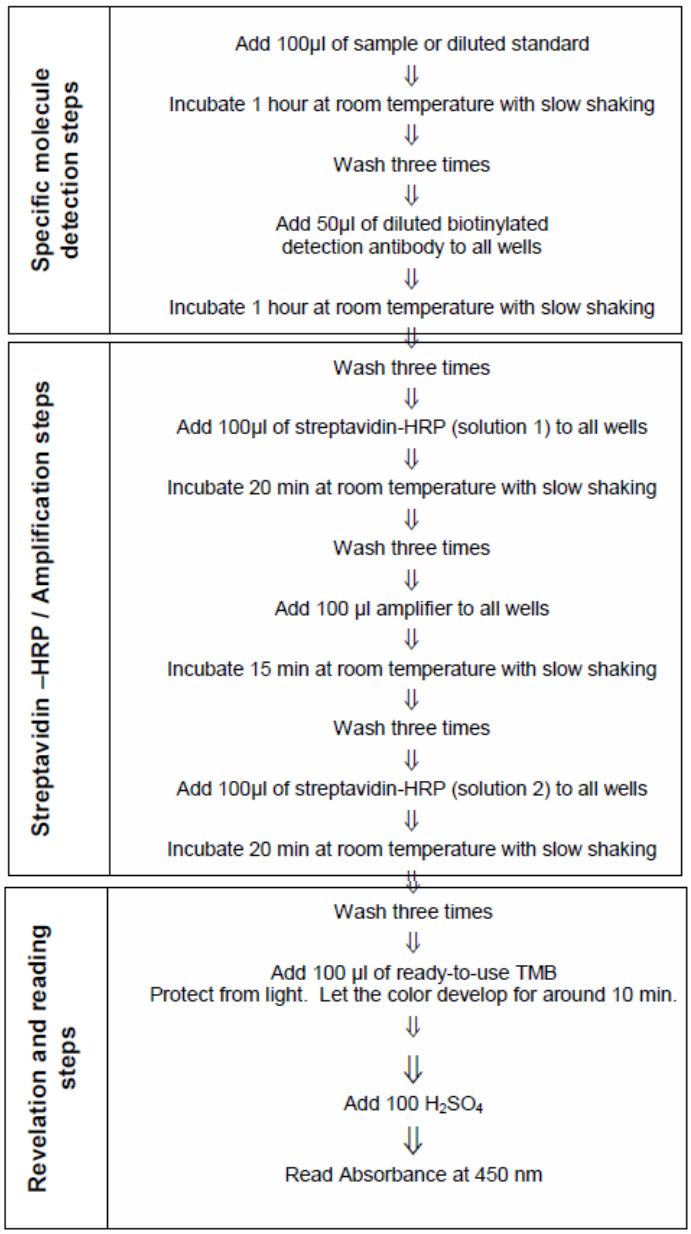
Storage Stability
Aliquots of spiked serum samples were stored at –20°C, 2-8°C, room temperature (RT) and at 37°C and the IL-10 level determined after 24h. There was no significant loss of IL-10 reactivity during storage at RT, and 2-8°C, however we observe 70% loss of reactivity when stored at 37°C if spiked in serum and 50% loss if spiked into culture media.

Freeze-thaw Stability
Aliquots of spiked serum were stored frozen at –20°C and thawed up to 5 times and the IL-10 level was determined. There was no significant loss of IL-10 after 5 cycles of freezing and thawing.

Standard Calibration
This immunoassay was calibrated against the International Reference Standard NIBSC 93/722. NIBSC 93/722 was quantitated in International Units (IU). 1 IU corresponded to 540 pg calibration IL-10.

12. Assay Summary

Total procedure length: 3 h 00 min



NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.