Human IL-8 ELISA Kit

Cat#: orb506698 (Product Manual)

Human IL-8 ELISA KIT

1. Intended use

The Biorbyt IL-8 ELISA kit is a solid phase sandwich ELISA for the in-vitro qualitative and quantitative determination of IL-8 in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant human IL-8.

This kit has been configured for research use only. Not suitable for use in therapeutic procedures.

2. Introduction

2.1. Summary

Interleukin 8 (IL-8) or CXCL8, Monocyte-Derived Neutrophil Chemotactic Factor (MDNCF), Neutrophil Activating Factor (NAF) and NAD-P1 is a chemokine secreted by monocytes, macrophages and endothelial cells. Interleukin 8 is a member of the CXC family, it plays a role as attractor and activator for neutrophils (1, 2, 3).

The predominant form of IL-8 is a 8.4kDa protein containing 72 amino acid residues, which includes five additional N-Terminal amino-acids. IL-8 contains the four conserved cysteine residues present in CXC chemokines and also contains the “ELR” motif common to CXC chemokines that binds to CXCR1 and CXCR2 (3, 4).

Data indicate that IL-8 plays a role in acute inflammation (13) and is implicated in the pathogenesis of rheumatoid arthritis (5, 6) and psoriasis (7).

Several studies have shown the implication of IL-8 in cancer progression through its mitogenic, angiogenic and metastatic effects (8). A high level of IL-8 in serum and plasma was described in different cancer such as recurrent breast cancer (9), colorectal cancer (10), prostate cancer (11, 14, 15) and ovarian cancer (12). Therefore, IL-8 was described as a potential marker for cancer progression and malignancy (8, 16). It was also described as a marker in specific type of obesity in combination with other cytokines such as IL-10 and IFNγ (17).

Due to its biological properties and its implication in cancer, this cytokine could be a target in treatment of cancer (8, 18, 14, 19, 20).

2.2. Principle of the method

A capture antibody highly specific for IL-8 has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of IL-8 samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-IL-8 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed.

The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing.

A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IL-8 present in the samples and standards.

The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL-8 in any sample tested.

3. Reagents provided and reconstitution

Reagents (Store@2-8^{0}C)	**Quantity** **1x48 well kit** Cat no. 950.050.048	**Quantity** **1x96 well kit** Cat no. 950.050.096	**Quantity** **2x96 well kit** Cat no. 950.050192	**Reconstitution**
96 well microtiter strip plate	1/2	1	2	Ready to use (Pre-coated)
Plastic plate covers	2	2	4	n/a
IL-8 Standard: 2000 pg/ml	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Standard Diluent (Buffer)	1 (25ml)	1 (25ml)	1 (25ml)	10x Concentrate, dilute in distilled water (see reagent preparation, section 8)
Standard Diluent Serum	1 (7 ml)	1 (7 ml)	2 (7 ml)	Ready to use
IL-8 Control	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Biotinylated anti-IL-8	1 (0.4ml)	1 (0.4ml)	2 (0.4ml)	Dilute in biotinylated antibody diluent (see reagent preparation, section 8)
Biotinylated Antibody diluent	1 (7ml)	1 (7ml)	1 (13ml)	Ready to use
Streptavidin-HRP	1 (5µl)	2 (5µl)	4 (5µl)	Add 0.5ml of HRP diluent prior to use (see reagent preparation, section 8)
HRP Diluent	1 (23ml)	1 (23ml)	1 (23ml)	Ready to use
Wash Buffer	1 (10ml)	1 (10ml)	2 (10ml)	200x Concentrate dilute in distilled water (see reagent preparation, section 8)
TMB Substrate	1 (11ml)	1 (11ml)	1 (24ml)	Ready to use
H_2SO_4 stop reagent	1 (11ml)	1 (11ml)	2 (11ml)	Ready to use

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- 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). 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 1X: Once prepared, store at 2-8°C for up to 1 week.

Standard diluent Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Reconstituted Standard/Control: Once prepared use immediately and do not store.

Diluted Biotinylated Anti-IL-8: Once prepared use immediately and do not store.

Diluted Streptavidin-HRP: Once prepared use immediately and do not store.

6. Specimen collection, processing & storage

Cell culture supernatants, human 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: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following 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 (250-500µl) to avoid repeated 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 haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use 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.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H2SO4 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 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 H2SO4 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 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, zero and control 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 2-8oC storage.

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

	Standards / Controls		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	2000	2000										
B	1000	1000										
C	500	500										
D	250	250										
E	125	125										
F	62.5	62.5										
G	zero	zero										
H	Ctrl	Ctrl										

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 Wash 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 may include 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 - Serum

For cell 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 2000 pg/ml of IL-8. 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 2000 to 62.5 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200µl of the reconstituted standard to well's A1 and A2, which provides the highest concentration standard at 2000 pg/ml.
- Add 100µl of standard diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100µl 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 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 2000 pg/ml to 62.5 pg/ml.
- Discard 100µl from the final wells of the standard curve (F1 and F2).

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

8.5. Preparation of Controls

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to your samples.

For serum and plasma samples : use Standard Diluent - Serum
For cells culture supernatants : use Standard Diluent Buffer

The supplied Controls must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution at the concentration stated on the vial. Do not store after use.

8.6. Preparation of Biotinylated anti-IL-8

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-IL-8 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
24	60	1590
32	80	2120
48	120	3180
96	240	6360

8.7. Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5µl vial with 0.5ml of HRP diluent immediately before use. 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:

Number of wells required	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.

Prepare all reagents as shown in section 8.

Note: final preparation of Biotinylated Secondary Antibody (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

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Assay Step		Details
1.	Addition	**Prepare Standard curve** as shown in section 8.4 above and transfer in the assay plate
2.	Addition	Add 100µl of each **Sample, Control and zero (Standard diluent)** in duplicate to appropriate number of wells
3.	Addition	Add 50µl of diluted **biotinylated anti-IL-8** to all wells
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour**
5.	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
6.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **30 min**
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
10.	Incubation	Incubate in the dark for **12-15 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100µl 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 620 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.

10. Data Analysis

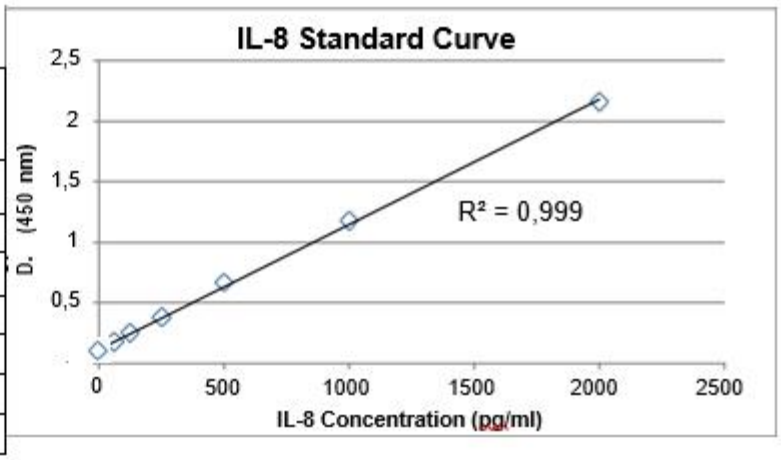
Calculate the average absorbance values for each set of duplicate standards, controls and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-8 standard concentration on the horizontal axis.

The amount of IL-8 in each sample is determined by extrapolating OD values against IL-8 standard concentrations using the standard curve.

Example IL-8 Standard curve

Standard	IL-8 Concentration (pg/ml)	OD (450nm) mean	CV (%)
1	2000	2.154	1.22
2	1000	1.173	1.03
3	500	0.663	2.53
4	250	0.378	5.16
5	125	0.249	4.01
6	62.5	0.174	5.79
zero	0	0.086	7.55



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. 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 Wash Buffer, fill with Wash 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. Performance Characteristics

12.1. Sensitivity

The sensitivity or minimum detectable dose of IL-8 using this Biorbyt IL-8 ELISA kit was found to be 29pg/ml. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 30 times.

12.2. Specificity

The assay recognizes both natural and recombinant human IL-8. To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested: IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IFNγ et TNFα.

12.3. Precision

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of samples containing different concentrations of IL-8: 3 in supernatant and 2 in human pooled serum. 1 standard curve was run on each plate. Data below show the mean IL-8 concentration and the coefficient of variation for each sample.
The calculated overall coefficient of variation was 2.5%.

Session	*Sample*	**Mean** IL-8 pg/ml	SD	CV%
Session 1	***Sample 1***	1850.33	25.11	**1.4**
	Sample 2	1166.67	13.01	**1.1**
	Sample 3	845.67	32.96	**3.9**
	Sample 4	1663.33	29.28	**1.8**
	Sample 5	995.00	6.00	**0.6**
Session 2	***Sample 1***	1835.33	81.24	**4.4**
	Sample 2	1096.67	12.42	**1.1**
	Sample 3	817.67	43.43	**5.3**
	Sample 4	1639.33	53.13	**3.2**
	Sample 5	932.33	32.75	**3.5**
Session 3	***Sample 1***	1740.00	28.62	**1.6**
	Sample 2	903.33	4.73	**0.5**
	Sample 3	797.67	32.32	**4.1**
	Sample 4	1579.33	23.69	**1.5**
	Sample 5	921.33	25.54	**2.8**

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) of samples containing different concentrations of IL-8: 3 in supernatant and 2 in human pooled serum. 1 standard curve was run on each plate. Data below show the mean IL-8 concentration and the coefficient of variation for each sample.

The calculated overall coefficient of variation was 9.7%.

	Sample 1	***Sample 2***	***Sample 3***	***Sample 4***	***Sample 5***
Mean IL-8 pg/ml	1682	1004	768	1497	880
SD	152	111	69	149	83
CV%	**9.0**	**11.0**	**8.9**	**9.9**	**9.4**

12.4. Dilution Parallelism

In two independent experiments two spiked human serum samples with different levels of IL-8 were analysed at different serial two fold dilutions (1:2 to 1:8) with two replicates each. Recoveries ranged from 70 to 105% with an overall mean recovery of 87%.

12.5. Spike Recovery

The spike recovery was evaluated by spiking 3 concentrations of IL-8 in human serum and culture medium in 3 separate experiments. Recoveries ranged from 96 to 110% with an overall mean recovery of 102%.

12.6. Stability

Storage Stability

Aliquots of spiked serum and spiked medium were stored at –20°C, +2-8°C, room temperature (RT) and at 37°C and the IL-8 level determined after 24h. A slight loss of IL-8 reactivity was observed at RT (11%) and 37°C (14%).

Freeze-thaw Stability

Aliquots of spiked serum and spiked medium were stored frozen at –20°C and thawed up to 5 times and the IL-8 level was determined. There was a significant loss of IL-8 reactivity after 5 cycles of freezing and thawing (18%).

12.7. Expected serum values

A panel of 16 human sera was tested for IL-8. 16 were below the detection level of 29pg/ml. Two samples reported results of 143 pg/ml and 197 pg/ml.

12.8. Standard Calibration

This immunoassay is calibrated against the International Reference Standard NIBSC 89/520. NIBSC 89/520 is quantitated in International Units (IU) and equivalence in ng/ml is indicated. It has been calculated that 1IU NIBSC (approximately 1ng) correspond to 1ng Biorbyt IL-8.

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15. Assay Summary

Total procedure length: 1h45min

Add 100µl of sample, controls and diluted standard
and 50µl Biotinylated anti-IL-8

↓

Incubate 1 hour at room temperature
↓

Wash three times
↓

Add 100µl of Streptavidin-HRP
↓

Incubate 30 min at room temperature
↓

Wash three times
↓

Add 100µl of ready-to-use TMB Protect from light. Let the color develop for 12-15 min. ↓

Add 100µl H2SO4

↓

Read Absorbance at 450 nm

16. International Summaries

16.1. French

PREPARATION DES REACTIFS : RESUME

1. Tampon de Lavage *(Wash Buffer)*

Ajouter 10 ml de **Tampon de Lavage concentré** (*Wash Buffer Concentrate*) 200 fois (200X) à 1990 ml d'eau distillée

2 Tampon de Dilution du Standard *(Standard Diluent Buffer)*

Ajouter 25 ml de **Tampon de Dilution du Standard concentré** 10 fois (*Standard Diluent Buffer Concentrate* 10X*)* à 225 ml d'eau distillée

3. Standard IL-8 *(IL-8 Standard)*

Reconstituer le **Standard IL-8** en ajoutant la quantité indiquée sur le flacon de Tampon de Dilution du Standard approprié

4. Contrôle IL-8 *(IL-8 Control)*

Reconstituer le **Contrôle IL-8** en ajoutant la quantité indiquée sur le flacon de Tampon de Dilution du Standard approprié

5. Anti-IL-8 Biotinylé *(Biotinylated anti-IL-8)*

Nombre de barrettes	**Anti-IL-8 Biotinylé Concentré (µl)**	**Diluent de l'Anticorps Biotinylé** (µl)
2	40	1060
3	60	1590
4	80	2120
6	120	3180
12	240	6360

6. Streptavidine-HRP (*Streptavidin-HRP*)

Nombre de barrettes	**Streptavidine-HRP pré-diluée** (µl)	**Diluent HRP** (ml)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

RESUME DU PROTOCOLE OPERATOIRE: durée totale : 1h45min

1. Ajouter 100µl de Tampon de Dilution du Standard approprié (Standard Diluent Buffer / Standard Diluent Serum), en duplicat, dans les puits Standards (B1 à F2).

2. Ajouter à la pipette 200µl de Standard IL-8 (IL-8 Standard) reconstitué dans les puits A1 et A2 puis réaliser des dilutions du Standard allant de 2000 à 62,5 pg/ml en transférant 100µl d'un puits à l'autre. Jeter les 100µl des derniers puits (F1 et F2).

3. Ajouter 100µl de Tampon de Dilution du Standard approprié (Standard Diluent Buffer / Standard diluent Serum) en duplicat dans les puits "blancs".

4. Ajouter 100µl d'échantillon (Sample), en duplicat, dans les puits désignés et 100µl de contrôle (IL-8 control), en duplicat dans les puits contrôles.

5. Préparer l'anticorps anti-IL-8 Biotinylé (Biotinylated anti IL-8).

6. Ajouter 50µl d'anticorps anti-IL-8 Biotinylé dilué (diluted biotinylated anti IL-8) dans tous les puits.

7. Couvrir les barrettes de puits et incuber pendant 1 heure à température ambiante (18-25°C).

8. Vider et laver les puits 3 fois avec le Tampon de Lavage (Wash Buffer).

9. Préparer la Streptavidine-HRP.

10. Ajouter 100µl de Streptavidine-HRP diluée (diluted Streptavidin-HRP) dans tous les puits.

11. Couvrir les puits et incuber pendant 30 minutes à température ambiante (18-25°C).

12. Vider et laver les puits 3 fois avec le Tampon de Lavage (Wash Buffer).

13. Ajouter 100µl de solution de TMB (TMB solution) prête à l'emploi dans tous les puits y compris les "blancs".

14. Incuber pendant environ 10-15 minutes à température ambiante (18-25°C) à l'obscurité.

15. Ajouter 100µl d'H2SO4 : Solution Stop (H2SO4 : Stop Reagent) dans tous les puits y compris les "blancs".

16. Mesurer l'absorbance (Densité Optique = D.O.) à la longueur d'onde 450 nm et optionnellement à 620 nm (entre 610 et 650 nm) comme longueur d'onde de référence.

Remarque: Les échantillons présentant une valeur de D.O. excédant la gamme de la courbe Standard peuvent résulter à des taux de IL-8 incorrects. C'est pourquoi, il est recommandé de diluer de tels échantillons avec le Tampon de Dilution du Standard (Standard Diluent Buffer) afin de quantifier précisément le véritable taux de IL-8.

16.2. Spanish

PREPARACIÓN DE LOS PRODUCTOS

1. Tampón de Lavado (Wash Buffer)

Añadir Tampón de Lavado Concentrado Concentrate) a 1990 ml de agua destilada.

200X (10 ml) (Wash Buffer

2 Tampón diluyente del estándar (Standard diluent buffer concentrate 10X) a 225 ml de agua destilada. (Standard Diluent Buffer)

Añadir Tampón Diluyente del Estándar Concentrado 10X (25 ml)

3. Estándar IL-8 *(IL-8 Standard)*	Reconstituir el **Estándar IL-8** añadiendo el Diluyente del Estándar appropriado, como indica la etiqueta del vial.		
4. Controles IL-8 *(IL-8 Control)*	Reconstituir los **Controles IL-8** añadiendo el Diluyente del Estándar appropriado, como indica la etiqueta del vial.		
5. Anti-IL-8 biotinilado *(Biotinylated anti-IL-8)*	Número de tiras	**Anticuerpo biotinilado concentrado** (µl)	**Diluyente del anticuerpo biotinilado** (µl)
	2	40	1,060
	3	60	1,590
	4	80	2,120
	6	120	3,180
	12	240	6,360
6. Estreptavidina-HRP *(Streptavidin-HRP)*	Número de tiras	**Estreptavidina-HRP prediluida** (µl)	**Diluyente de HRP** (ml)
	2	30	2
	3	45	3
	4	60	4
	6	75	5
	12	150	10

RESUMEN DEL PROTOCOLO. El procedimiento total tiene una duración de 1h45min.

1. Añadir 100µl del Tampón Diluyente del Estándar appropriado (Standard Diluent Buffer / Standard diluent Serum), por duplicado, a los pocillos designados para el estándar (B1 to F2).

2. Pipetear 200µl del Estándar IL-8 (IL-8 Standard) reconstituido en los pocillos A1 y A2 y hacer diluciones seriadas del estándar con el rango de concentraciones de 2000 al 62.5 pg/ml, transfiriendo 100µl de un pocillo al siguiente. Descartar 100µl de los últimos pocillos.

3. Añadir 100µl del Tampón Diluyente del Estándar appropriado (Standard Diluent Buffer / Standard diluent Serum), por duplicado, a los pocillos que van a ser el “blanco”.

4. Añadir 100µl de las muestras, por duplicado, a los pocillos designados para ello, y 100µl del Control reconstituido (IL-8 Control), por duplicado, a los pocillos designados como “control”.

5. Preparar el anticuerpo Anti-IL-8 Biotinilado (Biotinylated anti-IL-8).

6. Añadir 50µl del anti-IL-8 Biotinilado y diluido, a todos los pocillos.

7. Cubrir la placa e incubar durante 1 hora a temperatura ambiente (18-25°C).

8. Vaciar y lavar la placa 3 veces con Tampón de Lavado (Wash Buffer).

9. Preparar la Estreptavidina-HRP (Streptavidin-HRP).

10. Añadir 100µl de Estreptavidina-HRP diluida a todos los pocillos.

11. Cubrir la placa e incubar 30 minutos a temperatura ambiente (18-25°C).

12. Vaciar y lavar la placa 3 veces con Tampón de Lavado.

13. Añadir 100µl de solución TMB preparado para utilizar (TMB Substrate), a todos los pocillos, incluidos los pocillos con “blancos”.

14. Incubar la placa durante 10-15 minutos a temperatura ambiente (18-25°C) y en oscuridad.

15. Añadir 100µl de H2SO4 : Solución de Parada (H2SO4 : Stop Reagent), a todos los pocillos, incluidos los pocillos con los “blancos”.

16. Medir la intensidad de color (densidad óptica) a 450 nm y a 620 nm como longitud de onda de referencia (de 610 nm a 650 nm sería aceptable).

Nota: El cálculo de concentraciones de muestras con densidad óptica que supere el rango de la curva estándar, resultaría incorrecto, dando niveles de IL-8 más bajos de lo real. Estas muestras, requerirían ser diluidas con el Tampón de Dilución de Estándar, para poder precisar la cantidad real de IL-8.