Murine IL-13 ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 660.080.096

For research use only

Fast Track Your Research…….

Table of Contents

Murine IL-13 ELISA KIT

1. Intended use

The Diaclone Murine IL-13 ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of mIL-13 in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant Murine IL-13.

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

2. Introduction

2.1. Summary

Interleukin-13 (IL-13) is a Th2-type cytokine, secreted from CD4(+) T cells, mast cells, basophils and eosinophils. The IL-13 gene generates a cluster with other Th2-type cytokines such as IL-4 and IL-5. Although the homology between IL-13 and IL-4 at the amino acid level is low, the IL-13 structure determined by NMR is very similar to that of IL-4. Both cytokines share their receptors and signal pathways, giving these two cytokines similar biological properties.

IL-13 is an immunoregulatory cytokine. It has become evident that IL-13 is a key mediator in the pathogenesis of allergic inflammation. IL-13 mediates its effects by interacting with a complex receptor system comprised of IL-4Ralpha and two IL-13 binding proteins, IL-13Ralpha1 and IL-13Ralpha2.
IL-13 receptors are expressed on human B cells, basophils, eosinophils, mast cells, endothelial cells, fibroblasts, monocytes, macrophages, respiratory epithelial cells, and smooth muscle cells. However, functional IL-13 receptors have not been demonstrated on human or mouse T cells. Thus unlike IL-4, IL-13 does not appear to be important in the initial differentiation of CD4 T cells into T(H)2-type cells but rather appears to be important in the effector phase of allergic inflammation.

The important role of IL-13 in the pathogenesis of bronchial asthma as well as other allergic diseases has been recognized, based mainly on analyses of mouse models. Interleukin-13 further plays a major role in various other inflammatory diseases including cancer. The IL-13Ralpha2 but not IL-13Ralpha1 chain binds IL-13 with high affinity and is over expressed in a variety of human cancer cells derived from glioma, squamous cell carcinoma of head and neck, and AIDS-associated Kaposi's sarcoma.

2.2. Principle of the method

A capture Antibody highly specific for mIL-13 has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of mIL-13 samples and known standards to the capture antibodies and subsequent binding of the Biotinylated anti-mIL-13 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 mIL-13 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 mIL-13 in any sample tested.

3. Reagents provided and reconstitution

Reagents (Store@2-8°C)	**Quantity 1x96-well kit** Cat no. 660.080.096	**Reconstitution**
Anti-mIL-13 Coated Plate	1	Ready to use (96 well strip pre-coated plate)
Plastic plate covers	2	n/a
mIL-13 Standard	2	Reconstitute as directed on the vial (see Assay preparation, section 8)
Sample Diluent	1 (12ml)	Ready to use
Biotin Conjugate Anti-mIL-13	1 (70µl)	Dilute in Assay Buffer 1X (see Assay preparation, section 8)
Assay Buffer (PBS 1%Tween20 10%BSA)	1 (5ml)	20x concentrate dilute in distilled water (see Assay preparation, section 8)
Streptavidin-HRP	1 (150µl)	Dilute in Assay Buffer 1X (see Assay preparation, section 8)
Wash Buffer (PBS 1%Tween20)	1 (50ml)	20x concentrate dilute in distilled water (see Assay preparation, section 8)
Substrate Solution (TMB)	1 (15ml)	Ready to use
Stop Solution (1M Phophoric acid)	1 (15ml)	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)
- Microtiter plate 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 30 days.

Assay Buffer 1X: Once prepared, store at 2-8°C for up to 30 days.

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

Diluted Biotin Conjugate Anti-mIL-13: 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, Murine 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.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive mouse IL-13. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Do not thaw samples in a 37°C water bath. Do not vortex or sharply agitate samples.

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 Stop and Substrate Solutions. 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 Stop and Substrate solutions, 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 Substrate solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the Substrate solution with metal to prevent colour development. Warning Substrate 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 Substrate 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 Substrate 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 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		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1										
B	Std 2	Std 2										
C	Std 3	Std 3										
D	Std 4	Std 4										
E	Std 5	Std 5										
F	Std 6	Std 6										
G	zero	zero										
H												

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

8.2. Preparation of Wash Buffer 1X

Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water.
Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C.

8.3. Preparation of Assay Buffer 1X

Pour the entire contents (5 ml) of the Assay Buffer Concentrate (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 8°C.

8.4. Preparation of Standard

Standard vials must be reconstituted with the volume of distilled water shown on the vial immediately prior to use. This reconstitution gives a stock solution of 1000 pg/ml of mIL-13. 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 500 to 15.6 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 100µl of Sample Diluent to wells A1 and A2 to F1 and F2
- Add 100µl of reconstituted Standard mIL-13 to wells A1 and A2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- 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 500 pg/ml to 15.6 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 Biotin Conjugate

It is recommended this reagent is prepared immediately before use. Dilute the Biotin Conjugate Anti-mIL-13 with the Assay Buffer 1X in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of strips	Biotin Conjugate (µl)	Assay Buffer 1X (ml)
1 - 6	30	2.97
1 - 12	60	5.94

8.6. Preparation of Streptavidin-HRP

It is recommended this reagent is prepared immediately before use. Dilute the Streptavidin HRP with the Assay Buffer 1X in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of strips	Streptavidin-HRP (µl)	Assay Buffer 1X (ml)
1 - 6	60	5.94
1 - 12	120	11.88

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 Biotin Conjugate (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

Assay Step		Details
1.	Wash	a) Dispense 0.4 ml of **1x Wash Buffer** into each well b) Aspirate the contents of each well c) Repeat steps a and b Do not allow wells to dry before use
2.	Addition	**Prepare standard curve** as shown in section 8.4 above and add in duplicate to appropriate wells
3.	Addition	Add 100µl of each **zero (Sample Diluent)** in duplicate to appropriate number of wells
4.	Addition	Add 50µl of **Sample Diluent** to sample wells and 50µl of each **Sample** in duplicate to sample wells
5.	Addition	Add 50µl of diluted **Biotin Conjugate** to all wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hours** if available on a microplate shaker set at 100 rpm
7.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of **1x Wash Buffer** into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
8.	Addition	Add 100µl of diluted **Streptavidin-HRP** solution into all wells
9.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour** if available on a microplate shaker set at 100 rpm
10.	Wash	Repeat wash step 7.
11.	Addition	Add 100µl of **Substrate Solution** into all wells
12.	Incubation	Incubate in the dark for **10 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
13.	Addition	Add 100µl of **Stop Solution** into all wells
Read the absorbance value of each well (immediately after step 13.) 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 TMB substrate 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

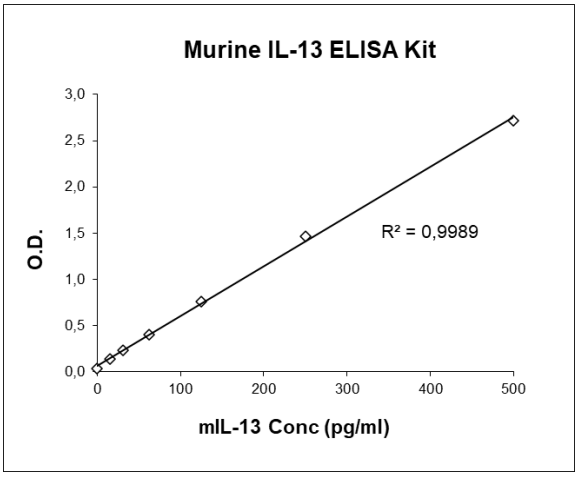
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding mIL-13 standard concentration on the horizontal axis.

The amount of mIL-13 in each sample is determined by extrapolating OD values against mIL-13 standard concentrations using the standard curve**.**

Example mIL-13 Standard curve

Standard	mIL-13 Conc (pg/ml)	OD (450nm) mean	CV (%)
1	500	2.721	1.3
2	250	1.469	0.8
3	125	0.757	3.3
4	62.5	0.404	0.9
5	31.3	0.236	1.5
6	15.6	0.141	4.2
zero	0	0.032	0.0



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.

For samples which have been diluted 1:2 according to the protocol, the calculated concentration should be multiplied by the dilution factor (x2).

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 Sample 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 mIL-13 using this Murine IL-13 ELISA kit was found to be **2.8 pg/ml**. This was determined by adding 2 standard deviations to the mean OD obtained when the zero standard was assayed in 6 independent experiments.

12.2. Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into serum. There was no cross reactivity detected, notably not with the inactive IL-13/IL-13BP complex.

12.3. Precision

Intra-assay
Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 5 serum and plasma samples containing different concentrations of mouse IL-13. 2 standard curves were run on each plate. Data below show the mean mouse IL-13 concentration and the coefficient of variation for each sample.
The calculated overall intra-assay coefficient of variation was 4.6%.

Inter-assay
Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 5 serum and plasma samples containing different concentrations of mouse IL-13. 2 standard curves were run on each plate. Data below show the mean mouse IL-13 concentration and the coefficient of variation calculated on 18 determinations of each sample.
The calculated overall inter-assay coefficient of variation was 5.0%.

12.4. Spike Recovery

The spike recovery was evaluated by spiking 4 levels of mouse IL-13 into serum and plasma samples. Recoveries were determined in 3 independent experiments with 6 replicates each.
The amount of endogenous product in unspiked serum was subtracted from the spike values.

The overall mean recovery of about 10% might be due to IL-13/IL13BP complex formation. (This inactive complex is not detected by this mouse IL-13 ELISA).

12.5. Stability

Storage Stability
Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the mouse IL-13 level determined after 24 h. There was no significant loss of mouse IL-13 immunoreactivity detected during storage under above conditions.

Freeze-thaw Stability
Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the mouse IL-13 levels determined. There was no significant loss of mouse IL-13 immunoreactivity detected by freezing and thawing.

12.6. Expected Values

A panel of sera and plasma samples from randomly selected apparently mice was tested for mouse IL-13. The detected mouse IL-13 sera levels ranged between 2.2 and 39.2 pg/ml with a mean level of 15.8 pg/ml. The detected mouse IL-13 plasma levels ranged between 0.0 and 13.3 pg/ml with a mean level of 4.2 pg/ml.

13. Assay Summary

Total procedure length: 3h10min

Add Samples, diluted Standards
and 50µl diluted Biotin Conjugate

↓

Incubate 2 hours at room temperature

↓

Wash three times

↓

Add 100µl of diluted Streptavidin-HRP

↓

Incubate 1 hour at room temperature

↓

Wash three times

↓

Add 100µl of Substrate Solution
Protect from light. Let the color develop for 10 min.

↓

Add 100µl of Stop Solution

↓

Read Absorbance at 450 nm

Supplier:

Diaclone SAS
6 Rue Dr Jean-François-Xavier Girod
25000 Besançon
France
Tel +33 (0)3 81 41 38 38
Email: techsupport@medixbiochemica.com