Human IL-13 Antibody Pair Kit

Module Set for the development of an ELISA for quantitative detection of human IL-13

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Read before opening

- Some vials contain small quantities of material, therefore centrifuge before use.
- This set of reagents is intended for use by persons experienced in the use of immunoassays. It is not suitable for use by inexperienced personnel.
- A sample protocol is included but please note that the protocol provided is a guideline. The type of substrate as well as all other reagents not included in the Module Set may influence assay performance.

Reagents provided

1 vial (1.1 mL) monoclonal Coating Antibody to human IL-13 (100 µg/mL)

1 vial (55 µL) Biotin-Conjugate anti-human IL-13 monoclonal antibody

1 vial (22 µL) Streptavidin-HRP

1 vial human IL-13 Standard protein lyophilized, 2000 pg/mL upon reconstitution

Storage instructions

Store kit components at -20°C. Immediately after use remaining reagents should be returned to -20°C storage. Avoid multiple freeze-thaw cycles. Aliquot reagents for repeated use at later dates. Reagents are labeled with expiration date.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IL-13.

Reagents and materials not provided

- Microwell plate
- Buffers and solutions (see "Preparation of buffers and solutions" on page 1 for preparation guidelines)

Precautions for use

All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) for specific advice.

Preparation of buffers and solutions

Note: The quality of BSA is a critical parameter for the test performance.

Phosphate buffered saline (PBS)

Reagents	Quantity
NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4 x 12 H_2O	2.85 g
KH_2PO_4	0.20 g
H_2O dest	adjust to 1 liter

Wash buffer

Add 0.5 mL Tween™ 20 to 1 liter of PBS and mix well.

Assay buffer (1x)

Reagents	Quantity
Bovine Serum Albumin (BSA)	5 g
Tween™ 20	0.5 mL
PBS	adjust to 1 liter

Fixing buffer

Reagents	Quantity
Sucrose	75 g
PBS	adjust to 500 mL

Substrate solution

1:2 mixture of H_2O_2 and Tetramethylbenzidine

Stop solution

1M Phosporic Acid (H_3PO_4)

Preparation of the microwell plate

Coating

1. Coating antibody final concentration is 1 µg/mL; 100 µL of the coating solution is added to each well. Dilute the coating antibody as following for one microtiter plate:

Reagents	Volume
PBS	10.890 mL
Coating antibody (100 µg/mL)	0.110 mL
Coating solution (1 µg/mL)	11.000 mL

2. Immediately after coating, seal the plate with an adhesive film and store at 2°C to 8°C over night, allowing the binding process to take place. Aspirate the contents of the wells and wash once with 400 µL of Wash Buffer according the washing procedure described in the test protocol below (see "Test protocol" on page 2).

Blocking and fixing

Blocking

Add 250 µL of Assay Buffer (1x) to each well and incubate at room temperature for 2 hours. Alternatively the plate may be blocked over night at 2°C to 8°C. Blocked plates can be stored at 2°C to 8°C for up to one week.

Fixing

To store the coated plates for longer than one week aspirate Assay Buffer (1x) and add 150 µL Fixing Buffer to each well. Incubate for 1 hour at room temperature. Aspirate Fixing Buffer and dry plates for over night at 28°C. When sealed with desiccant, the plates can be stored at 2°C to 8°C for 2 months.

Preparation of immunological reagents

Note: Centrifuge vials before opening to collect contents.

Preparation of standard

1. Reconstitute human IL-13 standard protein with deionized or distilled water. Reconstitution volume is indicated on the vial label (final concentration of reconstituted standard = 2000 pg/mL). Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
2. The concentrated human IL-13 standard must be diluted 1:10 with Assay Buffer (1x) just prior to use in a clean plastic test tube according to the following dilution scheme:

Reagents	Volume
conc. Standard Protein (2000 pg/mL)	30 µL
Assay Buffer (1x)	270 µL
Standard Protein (200 pg/mL)	300 µL

3. Shake gently to mix. After usage remaining diluted standard cannot be stored and has to be discarded.
4. Aliquot the reconstituted concentrated standard and store at -20°C.

Preparation of conjugate mixture

Dilute Biotin-Conjugate (1:1000) and Streptavidin-HRP (1:2500) with Assay Buffer (1x) before use. Use within 30 minutes after preparation. For one microwell plate dilute the stock reagents as follows:

Reagents	Volume
Biotin-Conjugate	5.5 µL
Streptavidin-HRP	2.2 µL
Assay Buffer (1x)	5492.3 µL
Conjugate Mixture	5500.0 µL

Test protocol

1. Wash blocked or blocked and fixed plates twice with approximately 400 µL Wash Buffer per well, with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell plate immediately after washing. Alternatively microwell plate can be placed upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.
2. Add 100 µL of Assay Buffer (1x) in duplicate to all standard wells. Pipette 100 µL of prepared standard (see “Preparation of standard“ on page 2), (concentration = 200 pg/mL) in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 pg/mL), and transfer 100 µL to wells B1 and B2, respectively. Mix the contents of wells B1 and B2 by repeated aspiration and ejection, and transfer 100 µL to wells C1 and C2, respectively. (see Figure 1). Take care not to scratch the surface of the microwells. Continue this procedure 4 times, creating two serially diluted columns of human IL-13 standard dilutions ranging from 100.0 to 1.6 pg/mL. Discard 100 µL from the last microwells (G1, G2). Final volume in all wells is 100 µL.

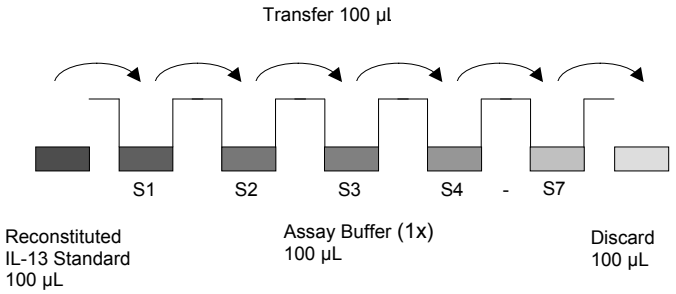

Fig. 1 Dilute standards - microwell plate

3. Add 100 µL of Assay Buffer (1x) in duplicate to blank wells.
4. Add 50 µL of Assay Buffer (1x) to the sample wells.
5. Add 50 µL of each sample in duplicate to the sample wells.
6. Prepare Conjugate Mixture (see “Preparation of conjugate mixture“ on page 2).
7. Add 50 µL of prepared Conjugate Mixture to all wells.
8. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 2 hours, on a microplate shaker if available.
9. Remove adhesive film and empty wells. Wash microwells 3 times according to point 1. of the test protocol. Proceed immediately to the next step.
10. Pipette 100 µL of Substrate Solution to all wells.
11. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

    Monitor the color development on the plate. The substrate reaction should be stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    Add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored on a plate reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

12. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added, or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
13. Read absorbance of each microwell on a spectro-photometer using 450 nm as primary wave length (you can use 620 nm as reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human IL-13 standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the y-axis, against the human IL-13 concentration on the x-axis. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of human IL-13 for each sample, first calculate the mean absorbance value for the duplicate wells of the sample, then extend a horizontal line from this point on the y-axis to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human IL-13 concentration.
- If instructions in this protocol have been followed samples have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- Calculation of samples with a concentration exceeding that of standard 1 may result in inaccurate, low human IL-13 levels (Hook Effect). Such samples require further external predilution according to expected human IL-13 values with Assay Buffer (1x) in order to precisely quantitate the actual human IL-13 level.
- Each testing facility should establish a control sample of known human IL-13 concentration and run this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

A basic understanding of immunoassay development and technical experience in ELISA performance are conditional for the successful use of this Module Set.

The protocol provided is just a guideline. The type of substrate as well as all other reagents not included in the Module Set may influence the test characteristics.

Human IL-13 module set characteristics

Specificity

The assay detects both natural and recombinant human IL-13. The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a IL-13 positive serum. No cross-reactivity was detected.

Expected values

A panel of 24 sera samples from randomly selected apparently healthy donors was were tested for human IL-13. The detected human IL-13 levels ranged between 0 and 44.4 pg/mL with a mean level of 8.2 pg/mL and a standard deviation of 12.1 pg/mL

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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