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ab273163
Human IL-31 ELISA kit

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Human IL-31 ELISA kit datasheet:
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For the in vitro qualitative and quantitative determination of IL-31 in supernatants, buffered solutions or serum and plasma samples and other body fluids.

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1. Overview

Human IL-31 ELISA kit (ab273163) for the in vitro qualitative and quantitative determination of IL-31 in supernatants, buffered solutions or serum and plasma samples and other body fluids. This assay will recognize both natural and recombinant human IL-31.

A capture Antibody highly specific for IL-31 has been coated to the wells of the microtitre strip plate provided during manufacture. Binding of IL-31 in samples and known standards to the capture antibodies is completed and then any excess unbound analyte is removed.

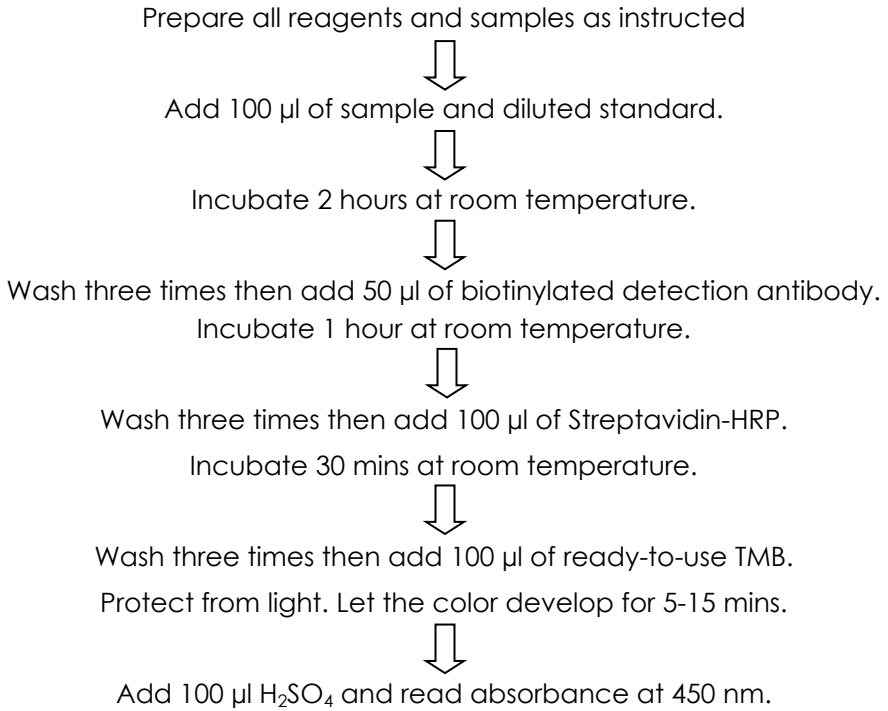
During the next incubation period the binding of the biotinylated anti-IL-31 secondary antibody to the analyte occurs. Any excess unbound secondary antibody is then removed.

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 colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of IL-31 present in the samples and standards.

The absorbance of the color 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-31 in any sample tested.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- 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.
- 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.

6. Materials Supplied

Item	Quantity	Storage Condition
IL-31 Coated Microwell strips	1 unit	+4°C
Plastic plate covers	2 units	+4°C
IL-31 Standard	2 vials	+4°C
Standard diluent Buffer	1 x 15 ml	+4°C
Biotinylated Antibody IL-31	1 x 400 µl	+4°C
Biotinylated Antibody diluent	1 x 7 ml	+4°C
Streptavidin-HRP	2 x 5 µl	+4°C
HRP Diluent	1 x 12 ml	+4°C
Wash Buffer	1 x 10 ml	+4°C
TMB Substrate	1 x 11 ml	+4°C
Stop Reagent	1 x 11 ml	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microtiter plate reader fitted with appropriate filters (450nm required with optional 620nm 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
- Miscellaneous laboratory plastic and/or glass, if possible sterile

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- To avoid high background always add samples or standards to the well before the addition of the antibody.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- **Wash Buffer 1X:** 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. Once prepared, store at 4°C for up to 1 week.
- **Standard Diluent Buffer 1X:** Add the contents of the vial (10x concentrate) to 225 ml of distilled water before use. Once prepared, store at 4°C for up to 1 week.
- **Diluted Biotinylated Anti-IL-31:** It is recommended this reagent is prepared immediately before use. Not store. Dilute the biotinylated anti-IL-31 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:

No. 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

- **Diluted 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 5 µl vial with 0.5 ml 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:

No. 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

- **Microwell Strips:** 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. 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 4°C storage.

10. Standard Preparation

- 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 1000 pg/ml of IL-31.
- 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 1000 – 31.25 pg/ml.
- A fresh standard curve should be produced for each new assay.

10.1 Immediately after reconstitution add 200 µl of the reconstituted standard to well's A1 and A2, which provides the highest concentration standard at 1000 pg/ml.

10.2 Add 100 µl of standard diluent to the remaining standard wells B1 and B2 to F1 and F2.

10.3 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.

10.4 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 1000 – 31.25 pg/ml.

10.5 Discard 100 µl from the final wells of the standard curve (F1 and F2).

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

11. Sample Preparation

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 mins.

- Serum or plasma samples have to be diluted 1:2 in Standard diluent.

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 mins and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 *x g* for 30 mins to remove particulates. Harvest plasma.

Δ Note: Before testing, serum or plasma samples have to be diluted 1:2 in standard diluent buffer.

Δ Note: If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) 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 hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Prepare Standard curve as shown in Section 10.
12.2 Add 100 µl of each Sample (diluted if applicable) and Standard diluent (zero) in duplicate to appropriate number of wells.
12.3 Cover with a plastic plate cover and incubate at room temperature (18-25°C) for 2 hours.
12.4 Remove the cover and wash the plate. Aspirate the liquid from each well. Dispense 0.3 ml of 1x washing solution into each well. Aspirate the contents of each well. Repeat another two times.
12.5 Add 50 µl of diluted biotinylated anti-IL-31 to all wells.
12.6 Cover with a plastic plate cover and incubate at room temperature (18-25°C) for 1 hour.
12.7 Repeat wash step 12.4.
12.8 Add 100 µl of Streptavidin-HRP solution into all wells.
12.9 Cover with a plastic plate cover and incubate at room temperature (18-25°C) for 30 min.
12.10 Repeat wash step 12.4.
12.11 Add 100 µl of ready-to-use TMB Substrate Solution into all wells.
12.12 Incubate in the dark for 5-15 mins at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.
12.13 Add 100 µl of H_2SO_4 Stop Reagent into all wells.
12.14 Read the absorbance value of each well (immediately after step 12.13) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength (610 nm to 650 nm is acceptable).

Δ Note: 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.

13. Calculations

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

13.2 Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-31 standard concentration on the horizontal axis.

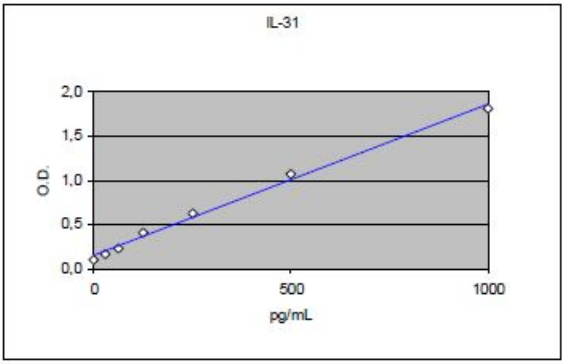
13.3 The amount of IL-31 in each sample is determined by extrapolating OD values against IL-31 standard concentrations using the standard curve.

13.4 For diluted samples 1:2 dilution, the calculated concentration should be multiplied by the dilution factor (x2)

Δ Note: 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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Example IL-31 Standard curve			
Standard	**IL-31 Conc**	**OD (450nm) mean**	**CV (%)**
1	1000	1.808	1.5
2	500	1.073	1.2
3	250	0.621	3.4
4	125	0.406	5.3
5	62.5	0.235	1.8
6	31.25	0.169	1.1
zero	0	0.106	0.6

Figure 1. Example of Human IL-31 ELISA kit standard curve.

15. Performance Characteristics

- **Sensitivity**

  The sensitivity or minimum detectable dose of IL-31 using this kit was found to be <18.3 pg/ml. This was determined by adding two standard deviations to the mean OD obtained when the zero standard was assayed 35 times (6 independent assays).

- **Specificity**

  The assay recognizes both natural and recombinant human IL-31. 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-1b, IL-2, Gp130, IL-4, IL-6, IL-6R, IL-17F, IL-27, IL-33, GM-CSF).

- **Precision**

  Intra-assay: Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) of 6 different samples containing different concentrations of IL-31. Data below show the mean concentration and the coefficient of variation for each sample. The overall intraassay coefficient of variation has been calculated to be 2.5%.

Session	Sample	Mean (pg/ml)	SD	CV%
1	Sample 1	1020.5	24.0	2.4
	Sample 2	461.9	18.9	4.1
	Sample 3	916.5	27.1	3.0
	Sample 4	469.3	8.7	1.9
	Sample 5	995.0	18.8	1.8
	Sample 6	494.2	16.2	3.3
2	Sample 1	1029.1	37.8	3.7
	Sample 2	515.0	6.6	1.3
	Sample 3	751.2	11.9	1.6
	Sample 4	391.8	7.8	2.0
	Sample 5	834.1	19.8	2.4
	Sample 6	409.4	6.1	1.5

3	Sample 1	1057.9	14.0	1.3
	Sample 2	476.8	10.4	2.2
	Sample 3	937.9	22.0	2.3
	Sample 4	498.2	17.9	3.6
	Sample 5	993.8	18.4	1.9
	Sample 6	492.4	22.9	4.6

Inter-assay: Assay to assay reproducibility within one laboratory will be evaluated in three independent experiments by two technicians. Each assay will be carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in RPMI and 2 in standard diluent with samples containing different concentrations of IL-31. 2 standard curves were run on each plate. The calculated overall coefficient of variation was 5.2%.

Sample	Mean (pg/ml)	SD	CV%
1	1078	58	5.4
2	508	30	6.0
3	887	33	3.7
4	472	25	5.3
5	966	40	4.1
6	490	32	6.5

- **Dilution Parallelism**

  Two spiked human serum with different levels of recombinant IL-31 were analyzed at three serial two fold dilutions (1:2-1:8) with two replicates each. Recoveries ranged from 66% to 126% with an overall mean recovery of 102%.

- **Spike Recovery**

  The spike recovery was evaluated by spiking three concentrations of recombinant IL-31 in human serum in two experiments. Recoveries ranged from 82% to 120% with an overall mean recovery of 98%.

- Stability

  Storage: Aliquots of spiked serum or culture media samples were stored at –20°C, 2-8°C, room temperature (RT) and at 37°C and the IL-31 level determined after 24h. There was no significant loss of IL-31 at 4°C for serum sample, and no significant loss at 4°C and RT for spiked culture media sample.

- Freeze-thaw: Aliquots of spiked serum or culture media were stored frozen at –20°C and thawed up to 5 times and IL-31

  level was determined. There was no significant loss of IL-31 reactivity during storage.

- Expected Values

  A panel of 20 plasmas and 20 serums of apparently healthy blood donors was tested for IL-31. All were below the detection level <18 pg/ml.

16. Notes

Technical Support

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