Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human IL-31 were tested 20 times on one plate, respectively.
Mean Intra-CV: 5.77%
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human IL-31 were tested on 3 different plates, 20 replicates in each plate.
Mean Inter-CV: 6.38%

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells between steps.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low OD values	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Sample values	Improper Sample Storage	Store the sample properly and use the fresh sample.
	Improper sample collection and preparation	Take proper sample collection and preparation method.
	Low quantity of analyte in samples	Use new sample and repeat assay.

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Human IL-31(Interleukin 31) ELISA Kit

Catalog No: tcee2234 96 Tests

Detection Range: 7.81-500pg/mL
Sensitivity: 4.69pg/mL
Specificity: No Significant cross-reactivity with analogues.
Storage: 4℃/-20℃
Validity: 6 months
Intended use:
It's used for in vitro quantitative determination of Human IL-31 concentrations in serum, plasma and other biological fluids.

Principle

The method to test Human IL-31 is typical Sandwich-ELISA. The plate was first pre-coated with Human IL-31 antibody. When standards or samples are combined with the coated antibody, the detection antibody which labeled with biotin combines to standard or samples next, then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively. The substrate solution TMB is used for color developing to appear blue. Finally the color changes to yellow by the acid(stop solution) function. The concentration of Human IL-31 is in direct proportion to the color shade which calculated by optical density.

Kit Components

The unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, take out the Micro ELISA Plate, Reference Standard, Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate to store at -20℃, the rest components are still stored at 4℃.
The volume of reagents is a little more than the volume marked on the label, please use in measuring instead of directly pouring.
All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Item	Size	Notice
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	4℃ for 1 month, -20℃ for 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	120 uL	
Concentrated HRP Conjugate (100×)	120 µL	4℃ for 1 month, -20℃ for 6 months (shading light)
Reference Standard & Sample Diluent	20 mL	4℃ for 6 months
Biotinylated Detection Ab Diluent	14 mL	
HRP Conjugate Diluent	14 mL	
Concentrated Wash Buffer (25×)	30 mL	
Substrate Reagent	10 mL	4℃ for 6 months (shading light)
Stop Solution	10 mL	
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Supplies required but not provided

Microplate reader with 450 nm wavelength filter

High-precision pipettes, EP tubes and disposable tips

37℃ Incubator

Loading slot for Wash Buffer

Deionized or distilled water

Absorbent paper

Sample Treatment

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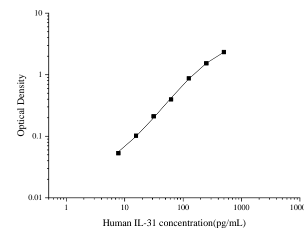
Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃, then centrifuge samples for 15 min at 1000×g at 2~8℃. Collect the supernatant for use. Note: Blood collection tubes should be disposable, non-endotoxin!

Typical data

Typical standard curve and data below is provided for reference only.

Concentration(pg/mL)	500	250	125	62.5	31.25	15.63	7.81	0
OD	2.403	1.603	0.943	0.468	0.282	0.173	0.124	0.071
Corrected OD	2.332	1.532	0.872	0.397	0.211	0.102	0.053	-



Recovery

The recovery of Human IL-31 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	93-107	99
EDTA plasma (n=5)	93-109	100
Cell culture media (n=5)	91-106	98

Linearity

Samples were spiked with high concentrations of Human IL-31 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)
1:2	Range (%)	96-110	85-99
	Average (%)	102	90
1:4	Range (%)	86-100	84-99
	Average (%)	93	91
1:8	Range (%)	93-104	85-96
	Average (%)	98	90
1:16	Range (%)	93-109	79-93
	Average (%)	99	85

5. **Reagent Preparation:** As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20～-80℃ and avoid repeated freezing and thawing.
6. **Reaction Time Control:** Please control reaction time strictly following this product description!
7. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.
8. **Stop Solution:** As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.
9. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.
10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
11. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).
12. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve on log-log graph paper, with the concentration on the x-axis and the OD values on the y-axis. Using some software such as OriginPro 8.0 can be also used to calculate the result.

If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

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Plasma: Collect plasma using EDTA-Na or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min. Collect the supernatant for use. Note: Hemolysis samples are not suitable for ELISA assay!

Tissue homogenates: The tissues should be minced to small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

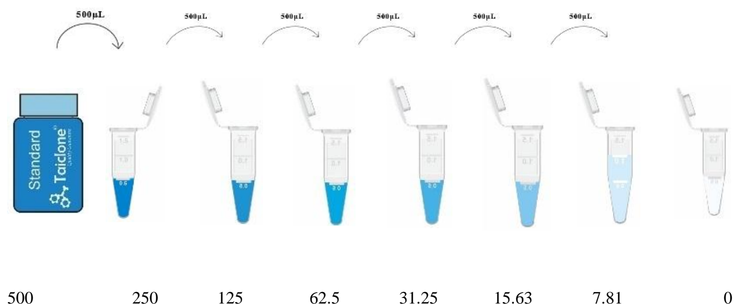
Other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~ 8℃. Collect the supernatant for use.

Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is above the most detection limit, end users must determine the optimal sample dilutions for their experiments. A preliminary experiment is suggested.
3. If the sample type is not included in the manual, also a preliminary experiment is suggested to verify the validity.
4. If lysis buffer is used to prepare tissue homogenate or cell culture supernatant, it may influence the testing result due to the introduced chemical substance.
5. Some recombinant protein may not be recognized due to the mismatching with antibodies used in the kit.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. When performing the assay, bring samples to room temperature (18-25℃).

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat the Microplate reader for 15 min before OD measurement.
2. **Standard working solution:** Centrifuge at 10,000×g for 1 minute, and dissolve the Standard with 1.0mL of Reference Standard &Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 500pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 500、250、125、62.5、31.25、15.63、7.81、0 pg/mL. If you want to make standard solution at the concentration of 250pg/mL working solution, you should take 500 μL of standard at 500pg/mL, add it to an EP tube with 500 μL of Reference Standard &Sample Diluent, and mix it. Procedures to prepare the remained concentrations are all the same. The Reference Standard &Sample Diluent serves as the zero (0 pg/mL).



3. **Biotinylated Detection Ab working solution:** Centrifuge the stock solution before use, dilute the stock solution to working solution with Biotinylated Detection Ab Diluent (1:99).
4. **Concentrated HRP Conjugate working solution:** Dilute the Concentrated HRP Conjugate to working solution with Concentrated HRP Conjugate Diluent (1:99).
5. **Wash Buffer:** Add 720 mL of deionized or distilled water to 30 mL of Concentrated Wash Buffer to prepare the wash working solution.

Note

1. The just opened ELISA Plate may appear water-like substance, which is normal and will not influence the experimental results.
2. Do not reuse the standard working solution, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solution should be stored back according to the storage condition in the above table.
3. Do not mix or use components from other lots (except for washing buffer and stop solution).
4. Change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent.

Plate Wash Method

1. **Wash manually:** Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper.
2. **Wash with a microplate washer:** Set parameters (liquid volume: 350uL; soaking time: 1~2 min; wash time: 3 or 5 times), put the plate to washer and start.

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Assay procedure

1. **Add Sample:** Add 100µL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃.
2. **Biotinylated Detection Ab:** Remove the liquid of each well, don't wash. Immediately add 100µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37 ℃.
3. **Wash:** Wash for 3 times.
4. **HRP Conjugate:** Add 100µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37 ℃.
5. **Wash:** Wash for 5 times.
6. **Substrate:** Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37 ℃. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, user should terminate the reaction.
7. **Stop:** Add 50µLof Stop Solution to each well. Then, the color turns to yellow immediately. The order to add stop solution should be the same as the substrate solution.
8. **OD Measurement:** Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm.

Note for assay procedure

1. **ELISA Plate:** The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
2. **Add Sample:** The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.
3. **Incubation:** To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
4. **Washing:** The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.