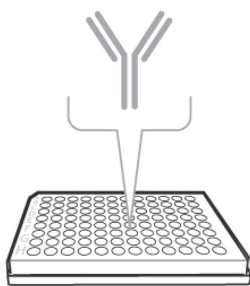
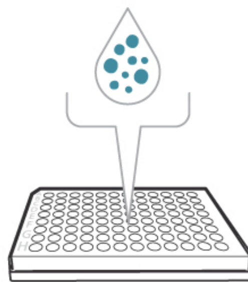
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

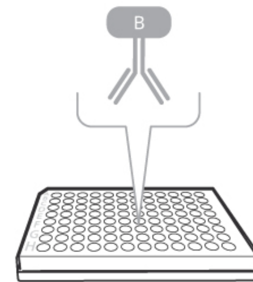
The Enzyme-Linked Immunosorbent Assay (ELISA) is an immunological technique used in biochemistry for the detection and quantification of antigens or antibodies. Depending on the intended purpose, different methods can be followed, all based on the ability to detect the antigen-antibody reaction using a second antibody capable of binding the first and conjugated with a particular enzyme (usually alkaline phosphatase or horseradish peroxidase) that catalyses a colour reaction with the addition of a suitable substrate. The development of colour is indicative of the presence of the antigen or antibody to be assayed and the intensity of the colouration can be measured using a spectrophotometer.



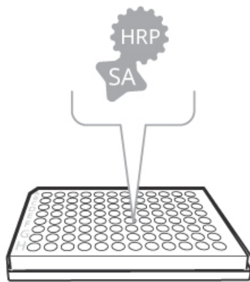
1. Coating



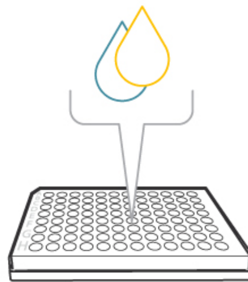
2. Analyte capture



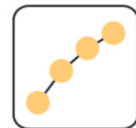
3. Detection antibody



4. Streptavidin-enzyme conjugate



5. Substrate addition



6. Analysis and calculation

Human IL-31 and IL-6 Detection

Components provided by the kit

- 96-well microplate
- Plate sealers
- Phosphate Buffered Saline (PBS) <u>filtered</u>, pH 7.2, 7.4
- Wash Buffer: 0.05% Tween-20 in PBS, pH 7.2, 7.4
- Reagent diluent <u>filtered</u>: 1% BSA in PBS, pH 7.2, 7.4
- Substrate solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine)
- Stop Solution: 2 N H_2SO_4

- Human IL-31/IL-6 Capture Antibody
- Human IL-31/IL-6 Detection Antibody
- Human IL-31/IL-6 Standard
- Streptavidin-HRP (horseradish peroxidase)

REAGENT PREPARATION

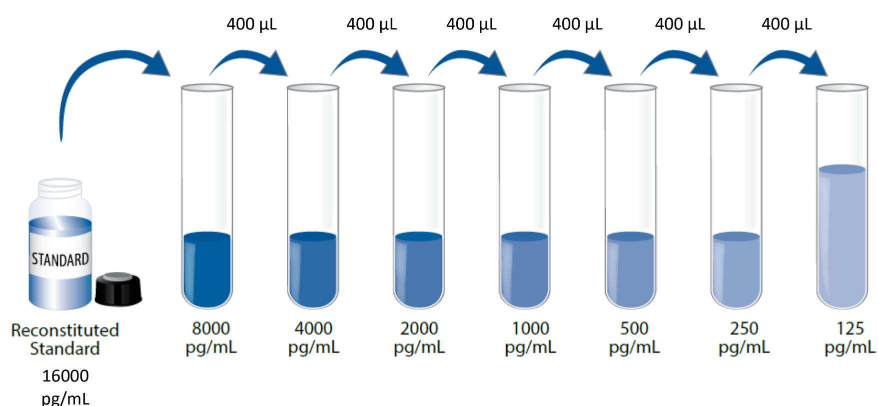
Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP: 1.0 mL of streptavidin conjugated to horseradishperoxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Goat Anti-Human IL-31 Capture Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of PBS. Dilute in PBS without carrier protein to the working concentration indicated on the C of A.

Biotinylated Goat Anti-Human IL-31 Detection Antibody: Refer to the lot-specific C of A for amount supplied. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A.

Recombinant Human IL-31 Standard: Refer to the lot-specific C of A for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven-point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended (400 µL from double-concentrated standard point + 400 µL Reagent Diluent).



STANDARD POINTS DILUTION		
Concentration (pg/mL)	**Standard Volume (mL)**	**Reagent Diluent Volume (mL)**
16000	0.5	/
8000	0.4	0.4
4000	0.4	0.4
2000	0.4	0.4
1000	0.4	0.4
500	0.4	0.4
250	0.4	0.4
125	0.4	0.4

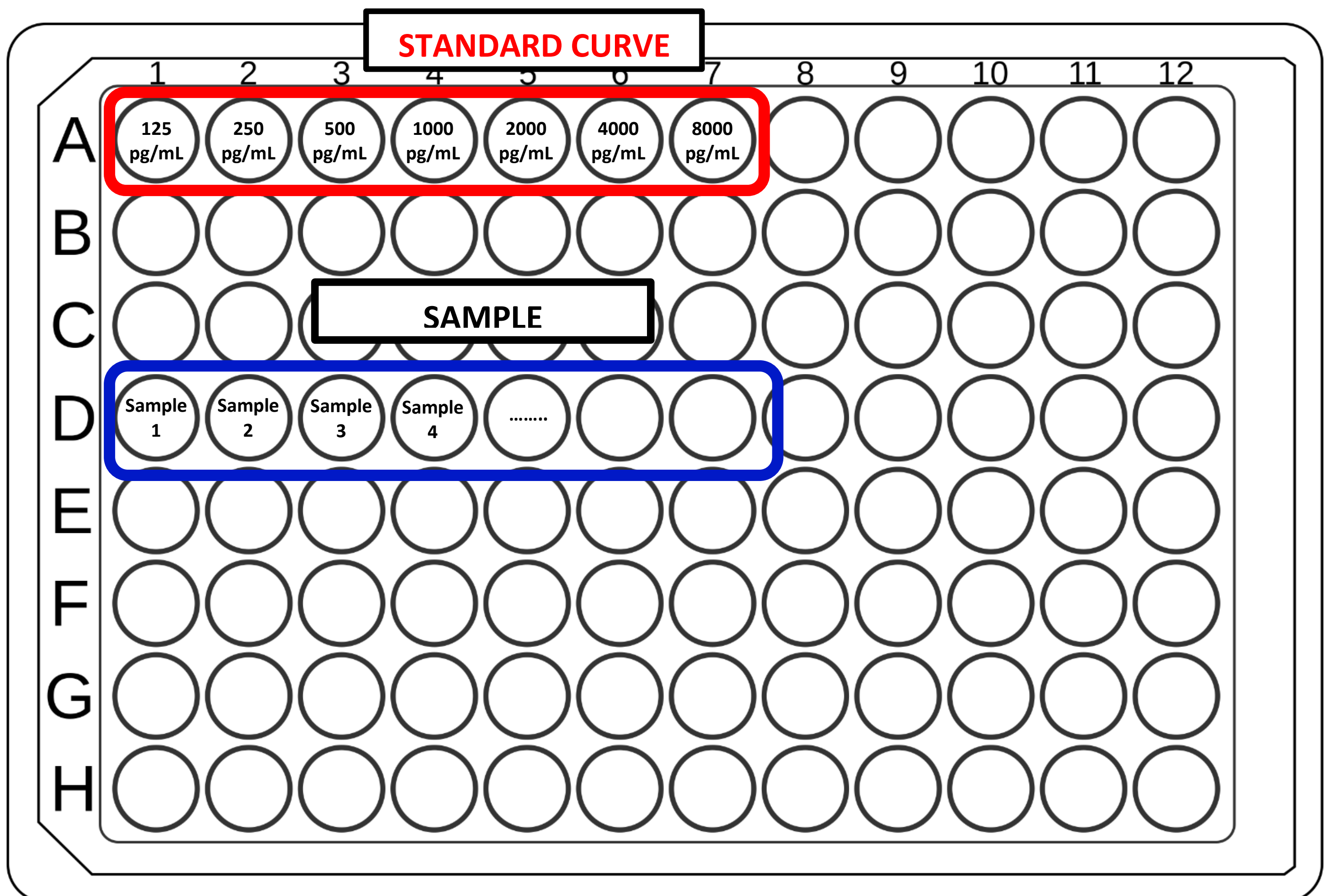
Plate preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.

2. Aspirate each well and wash with Wash Buffer, repeating the process for a total of two washes. Wash by filling each well with 300 µL of Wash Buffer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Block plates by adding 300 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 25 min.

4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.



Assay procedure

1. Add 100 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 30 min/1 h at room temperature.

2. Repeat the aspiration/wash as in step 2 of Plate Preparation.

3. Add 100 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 30 min/1 h at room temperature.

4. Repeat the aspiration/wash as in step 2 of Plate Preparation.

5. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

6. Repeat the aspiration/wash as in step 2.

7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Data analysis

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-31/IL-6 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical standard curve