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ab237669
Avelumab ELISA Kit

For the measurement of Avelumab in human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Avelumab ELISA Kit (ab237669) is a highly specific and sensitive kit designed for the in vitro determination of Avelumab in biological matrices such as human serum and plasma. The density of color is proportional to the amount of human Avelumab captured from the samples.

Avelumab is a fully human anti-PD-L1 IgG1 lambda monoclonal antibody that has a molecular weight of approximately 147 kDa. Avelumab binds PD-L1 and blocks the interaction between PD-L1 and its receptors PD-1 and B7-1. By inhibiting PD-L1 interactions, avelumab is thought to enable the activation of T-cells and the adaptive immune system. By retaining anative Fc-region, avelumab is thought to engage the innate immune system and may induce antibody-dependent cell-mediated cytotoxicity. Importantly, avelumab has not shown antibody-dependent cell mediated cytotoxicity against immune cell subsets in humans.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL of Assay buffer to each well. Add 10 µL of standard or sample to appropriate wells. Cover and incubate for 30 minutes at room temperature

Discard incubation solution and wash plate 3 times with 300 µL diluted Wash Buffer

Add 100 µL HRP-conjugate to each well. Cover and incubate for 30 minutes at room temperature

Discard the solution and wash plate 3 times with 300 µL diluted Wash Buffer

Add 100 µL TMB Substrate and incubate the plate in the dark at room temperature for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm within 20 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handle 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.

- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
  - 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. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

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.

6. Materials Supplied

Item	Quantity	Storage

		condition
Micro ELISA Plate	1 unit	+4°C
Avelumab Standard S1	0.3 mL	+4°C
Avelumab Standard S2	0.3 mL	+4°C
Avelumab Standard S3	0.3 mL	+4°C
Avelumab Standard S4	0.3 mL	+4°C
Avelumab Standard S5	0.3 mL	+4°C
Avelumab Standard S6	0.3 mL	+4°C
Avelumab Standard S7	0.3 mL	+4°C
Assay Buffer	2 X 50 mL	+4°C
HRP-conjugate Probe	12 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Wash Buffer (20X)	50 mL	+4°C
Plate sealers	2 units	+4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 450 nm
  - Deionized water.
  - Multi- and single-channel pipettes.
  - Tubes for sample dilution.
  - Plate shaker for all incubation steps.
  - Absorbent paper

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted.
- 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.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 20X Wash Buffer:

Dilute the 20X Wash Buffer to 1X solution in ddH_2O (10 mL of Wash Buffer stock to 190 mL of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

10. Standard and Control Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

Dilute standards 1:100 with Assay Buffer (10 µl Sample + 990 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7
Stock conc. µg/mL	300	100	30	10	0	High control	Low control
Working conc. ng/mL	3000	1000	300	100	0	-	-

Concentration for high and low controls are indicated on vials.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

11.1 Serum/plasma:

- Dilute samples at 1:100 (10 µl Sample + 990 µl Assay Buffer).
- Dilute samples at 1:100 (10 µl Sample + 990 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Avelumab is higher than the measuring range.
- Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

Δ Note: The usual precautions for venipuncture should be observed.

12. Assay Procedure

- Prepare reagents within 30 minutes before the experiment.
- Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Pipette 100 µl of Assay Buffer non-exceptionally into each of the wells to be used.

12.2 Add 10 µL of standards and diluted samples into appropriate wells. Cover wells and incubate for 30 minutes at room temperature.

12.3 Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.

12.4 Add 100 µL of HRP-conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 30 minutes.

12.5 Discard the solution and wash the wells as step 12.3.

12.6 Add 100 µL of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 10 minutes.

12.7 Add 100 µL of Stop solution to stop the reaction.

12.8 Read the absorbance in a microplate reader set to 450 nm within 20 minutes. (Reference wavelength to 650 nm).

13. Calculations

13.1 Calculate the average absorbance value for the blank control (0 ng/mL) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

13.2 Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard.

13.3 Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit.

13.4 To obtain the exact values of the samples, the concentration determined from the standard curve should be multiplied by the dilution factor.

13.5 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate dilution factor to obtain the concentration of target protein in the sample.

13.6 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

14. Typical Data

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

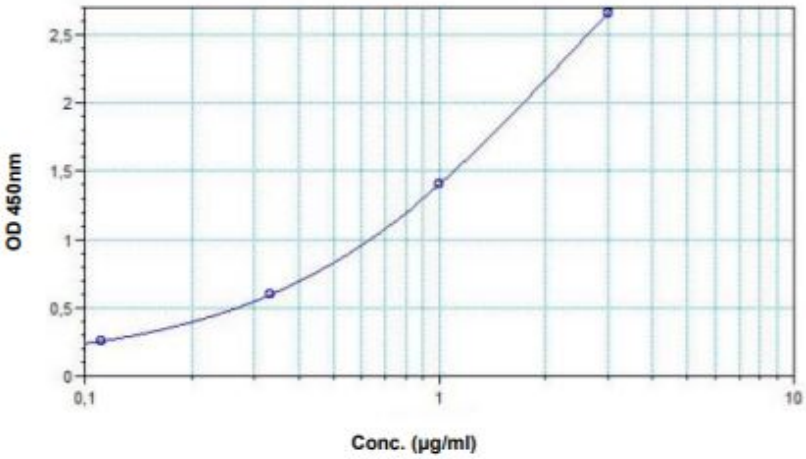

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

15. Typical Sample Values

Detection Range: 100 - 3000 ng/mL.

Sensitivity: 100 ng/mL.

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

Cross Reactivity: Except for Avelumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.
Recovery rate: <100±30% with normal human serum samples with known concentrations.

16. Notes

Technical Support

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