Anti-Avelumab (Bavencio®) ADA Quantitative ELISA

SKU: HUMB00043

Enzyme immunoassay for the qualitative determination of antibodies to avelumab (Bavencio®) in serum and plasma

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Required Volume (µl)	10
Total Time (min)	140
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	+/-
Spike Recovery (%)	-
Shelf Life (year)	1

Intended Use

The Elisa Genie® Antibody to avelumab (Bavencio®) Enzyme-Linked ImmunoSorbent Assay (ELISA) Kit is intended for the qualitative determination of antibodies to avelumab (Bavencio®) in serum and plasma. It is for professional use only.

Summary and Explanation

Avelumab (Bavencio®) (also known as MSB0010718C) is an investigational fully human anti-PD-L1 IgG1 lambda monoclonal antibody that has a molecular weight of approximately 147 kDa. By inhibiting PD-L1 interactions, avelumab is thought to enable the activation of T-cells and the adaptive immune system. By retaining a native Fc-region, avelumab is thought to engage the innate immune system and may induce antibody-dependent cell-mediated cytotoxicity (ADCC). Importantly, avelumab has not shown antibody-dependent cell-mediated cytotoxicity against immune cell subsets in humans.

Mechanism of Action

PD-L1 may be expressed on tumour cells and tumour-infiltrating immune cells and can contribute to the inhibition of the anti-tumour immune response in the tumour microenvironment. Binding of PD-L1 to the PD-1 and B7.1 receptors found on T cells and antigen presenting cells suppresses cytotoxic T-cell activity, T-cell proliferation and cytokine production. Avelumab binds PD-L1 and blocks the interaction between PD-L1 and its receptors PD-1 and B7.1. This interaction releases the inhibitory effects of PD-L1 on the immune response resulting in the restoration of immune responses, including anti-tumour immune responses. Avelumab has also been shown to induce antibody-dependent cell-mediated cytotoxicity (ADCC) in vitro. In syngeneic mouse tumour models, blocking PD-L1 activity resulted in decreased tumour growth.

Pharmacokinetics

The pharmacokinetics of avelumab was studied in 1629 patients who received doses ranging from 1 to 20 mg/kg every 2 weeks. The data showed that the exposure of avelumab increased dose-proportionally in the dose range of 10 to 20 mg/kg every 2 weeks. Steady-state concentrations of avelumab were reached after approximately 4 to 6 weeks (2 to 3 cycles) of repeated dosing, and the systemic accumulation was approximately 1.25-fold. According to FDA's data, Cmax value was found 301 µg/mL and Cmin value was found as 22 µg/mL. In observed ADA-positive patients, the Cmin value was increased to 27,2 µg/mL, while in ADA-negative patients, this value decreased to 14,1 µg/mL

Distribution

The geometric mean volume of distribution at steady state for a subject receiving 10 mg/kg was 4.72 L.

Elimination

The primary elimination mechanism of avelumab is proteolytic degradation. Based on population pharmacokinetic analyses in patients with solid tumours, the total systemic clearance was 0.59 L/day and the terminal half-life was 6.1 days in patients receiving 10 mg/kg. In a post hoc analysis, avelumab clearance was found to decrease over time in patients with MCC, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline value of approximately 41.7% (40.0%).

Specific populations

Body weight was positively correlated with total systemic clearance in population pharmacokinetic analyses. No clinically meaningful differences in pharmacokinetics were observed in the clearance of avelumab based on age; sex; race; PD-L1 status; tumor burden; mild [calculated creatinine clearance (CLcr) 60 to 89 mL/min, n=623 as

estimated by the Cockcroft-Gault formula], moderate [CLcr 30 to 59 mL/min, n=320] or severe [CLcr 15 to 29 ml/min, n=4] renal impairment; and mild [bilirubin less than or equal to ULN and AST greater than ULN or bilirubin between 1 and 1.5 times ULN, n=217] or moderate [bilirubin between 1.5 and 3 times ULN; n=4] hepatic impairment. There are limited data from patients with severe hepatic impairment [bilirubin greater than 3 times ULN, n=1], and the effect of severe hepatic impairment on the pharmacokinetics of avelumab is unknown.

Immunogenicity

As with all therapeutic proteins, there is potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to avelumab in the studies described below with the incidence of antibodies in other studies or to other products may be misleading. Of 1738 patients treated with BAVENCIO 10 mg/kg as an intravenous infusion every 2 weeks, 1558 were evaluable for treatment-emergent anti-drug antibodies (ADA) and 64 (4.1%) tested positive. The development of treatment-emergent ADA against avelumab did not appear to alter the pharmacokinetic profile or risk of infusion-related reactions.

The use of avelumab (Bavencio®) was associated to the development of anti-avelumab antibodies, even some might be neutralizing, in various percentages of patients during therapy with the drug. The Elisa Genie Avelumab-ELISA and Antibody to Avelumab ELISA Kits can be efficiently used, for monitoring serum through levels and the presence of anti-avelumab antibodies respectively, during therapy and offers the scientist a tool for decision on possible preventive measures.

Test Principles

The Elisa Genie® Antibody to avelumab (Bavencio®) ELISA is a sandwich assay for the determination of antibodies against avelumab in serum and plasma samples. During the first incubation period, antibodies to avelumab (ATAV) in patient serum/ plasma samples are captured by the drug avelumab (Bavencio®) coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled specific conjugate is added to each well and then incubated. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction colour is directly proportional to the concentration of ATAV in sample.

Warnings and Precautions

1. For professional use only and professional use only.

2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.

3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

6. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.

7. Avoid contact with Stop solution. It may cause skin irritations and burns.

8. All reagents of this kit containing human serum or plasma (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

Specimen Collection and Storage
Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observedq. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light
Stability:	7 d	6 mon	Avoid repeated freeze-thaw cycles

*. Avelumab (Bavencio®) infusion camouflages/masks the presence of antibody to avelumab (ATAV) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATAV. The Elisa Genie® Laboratories suggests to obtain blood sample just before the infusion of avelumab (Bavencio®) or at least 2 weeks after the infusion of avelumab (Bavencio®).

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with avelumab.
1 x 0.25 mL	RCTV CNTR	**Reactive Control** Ready-to-use. Contains Avelumab-reactive reagent, human serum, stabilizers and <0.1% NaN_3
1 x 0.5 mL	NEG CNTR	**Negative Control** Ready-to-use. Contains human serum, stabilizers and <0.1% NaN_3
1 x 12 mL	ASSAY BUF	**Assay Buffer** Blue coloured. Ready to use. Contains proteins and <0.1% NaN3
1 x 12 mL	POD CONJ	**Peroxidase Conjugate** Red coloured. Ready to use. Contains peroxidase (POD) conjugate, stabilizer and preservatives.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains TMB
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	**Wash Buffer, concentrate (20x)** Contains Buffer with Tween 20.
2 x 1	ADH FILM	**Adhesive Film** For covering of Microtiter Plate during incubation.

Materials Required but not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system.
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. ddH_2O or deionised water, paper towels, pipette tips and timer.

Procedure Notes

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Preparation of Component

Dilute/ dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	ddH_2O	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	2 w

*. Prepare Wash Buffer before starting assay procedure.

Test Procedure

1	Pipette 100µl of Assay Buffer non-exceptionally into each of the wells to be used.
2.	**QUALITATIVE ELISA TEST FORMAT** Pipette 10 µL of ready-to use Negative Control, Reactive Control, and Samples into the respective wells of microtiter plate Wells A1: Negative Control B1: Negative Control C1: Reactive Control D1 and so on: Sample (serum/plasma)
3	Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. Incubate 60 min at room temperature (18-25°C).
4	Remove adhesive film. 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.
5	Pipette 100 µL of ready-to use Peroxidase Conjugate into each well.
6	Cover the plate with adhesive film. Incubate 60 min at room temperature (18- 25°C).
7	Remove adhesive film. 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.
8	Pipette 100 µL of TMB Substrate Solution into each well.
9	Incubate 20 min (without adhesive foil.) at room temperature (18-25°C) in the dark.
10	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
11	Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

Interpretation of Results

For the run to be valid, the OD450 nm of Positive Control should be ≥ 1.00 and the OD450/650 nm of each Negative Control should be ≥ 1.00 and the $OD_{450/650}$ nm of each Negative Control should be <0.200 0, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD450/650nm of the negative controls by 3.

E.g.;

If "Sample OD450/650 the mean OD450 /650 of Negative Controls" is ≥3, the sample is POSITIVE

If "Sample OD450/650 the mean OD450/650 of Negative Controls" is <3, the sample is NEGATIVE