KRIBIOLISA™ Anti-Atezolizumab ELISA

REF : KBI2027

Ver 3.0

RUO

Enzyme Immunoassay for the Qualitative Determination of Anti-Atezolizumab in human serum and plasma

RUO	**For Research Use**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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Introduction:

Atezolizumab (trade name Tecentriq) is a fully humanized, engineered monoclonal antibody of IgG1 isotype against the protein programmed cell death-ligand 1 (PD-L1). In 2015, it was in clinical trials as an immunotherapy for several types of solid tumors. It was under investigation by Genentech/Roche. In April 2016, Roche announced that atezolizumab had been granted fast track status for lung cancer by the FDA.In May 2018, Tecentriq was in combination with Avastin and standard chemotherapy for some patients with lung cancer was granted priority review. Anti-Drug Antibodies (ADA) may induce unwanted side effects in biopharmaceuticals. Hence, ADA has been subjected to increase in scrutiny by the regulatory authorities using immunogenicity safety studies. ADA has been observed in pre-clinical and clinical studies, resulting in significant changes in toxicology, pharmacokinetics and efficacy. These effects result from the generation of drug-induced (neutralizing) autoantibodies against Atezolizumab and can be responsible for allergic reaction, or even anaphylactic shock. This ELISA kit detects antibodies for Anti-Atezolizumab and may be used for monitoring immunogenicity.

Intended Use:

The KRIBIOLISA™ Anti-Atezolizumab ELISA is used as an analytical tool for qualitative determination of Anti-Atezolizumab in human serum and plasma

Principle:

The method employs the competitive enzyme immunoassay technique. Atezolizumab is pre-coated onto microwells. Samples or controls along with the tracer are pipetted into microwells and antibodies to Atezolizumab present in the positive control or sample will compete with tracer and bound by the capture antibody. After washing microwells, HRP conjugate is pipetted and incubated. Free HRP conjugate will be removed by washing cycle. The ready to use substrate solution (TMB) is added to microwells and color develops inversely proportionally to the amount of Anti-Atezolizumab present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Atezolizumab Coated Microtiter Plate (12x8 wells) – 1 no
2. Anti Atezolizumab Positive Control (0.5 ml) - 1 vial
3. Anti-Atezollizumab Negative Control (0.5 ml) - 1 vial
4. Biotinylated Atezolizumab – 6 ml
5. Streptavidin:HRP Conjugate – 1 vial
6. Strep HRP conjugate diluent – 10ml
7. Sample Diluent – 60 ml
8. Wash Buffer (20X) – 25 ml
9. TMB Substrate – 12 ml
10. Stop Solution – 12 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Semi-Log graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.

3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation - Samples have to be diluted 1:10 (v/v), e.g. for 1:10 (1 ul sample + 9 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires the samples to be kept at -20°C.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room Temperature before use.
3. Add **7.5ul of Streptavidin HRP Conjugate** to **2992.5ul of Strep HRP Conjugate diluent** to make **3 ml Strep HRP Conjugate Ready to use solution**.
4. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.

Procedural Notes:

5. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
6. High Dose Hook Effect may be observed in samples with very high concentrations of Anti-Atezolizumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Atezolizumab present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Anti-Atezolizumab concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
7. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Atezolizumab.
8. It is recommended that all Controls and Samples be assayed in duplicates.
9. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
10. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
11. The plates should be read within 30 minutes after adding the Stop Solution.
12. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at Room Temperature.
2. Pipette **25 ul** of **Positive Control, Negative Control and Samples** to the respective wells.
3. Pipette **50 ul Biotinylated Atezolizumab** antigen to all the wells.
4. Cover the plate and incubate for 120 minutes at Room Temperature.
5. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Pipette **50 ul** of **Streptavidin:HRP Conjugate Ready to use solution** to all wells.
7. Cover the plate and incubate for 60 minutes at Room Temperature.
8. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
9. Add **100 ul** of **TMB Substrate** in each well.
10. Incubate the plate at Room Temperature for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
11. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate reader.

Interpretation of Results:

Calculation for Cut off Values
Read the sample and negative control wells on microtitre plate reader at 450nm. The OD (Optical Density) of NC (Negative Control) in duplicate should be used for calculating the mean and standard deviation. This is the $Negative_{mean}$.

The Cut-Off for Positives is equal to a value less than ($Negative_{mean}$ + 3*Standard Deviation).
Formula:
Positive Sample Value = OD < Cut-Off (**$Negative_{mean}$** ***+ 3*SD)***

For Positive Samples **< Cut Off**
For Negative Samples **>= Cut Off**

Validity of the Test:
The use of controls allows validation of the test. The results should not be used if a control is out of range. A run is valid if the following condition is met:
Positive Control Value < Cut Off

Typical example –

Sample Type	*Absorbance #1*	*Absorbance #2*	*Mean*
Negative	*1.131*	*1.128*	*1.129*
Standard Deviation	*1.131-1.129 = 0.002*	*1.128-1.129 = -0.001*	

Mean Standard Deviation = $\sqrt{(0.002)^2 + (-0.001)^2/2} = 0.0014$

*Therefore Cut-off = Mean + 3*SD*
= 1.129 + 3 0.0014*
= 1.129 + 0.0042
= 1.133
Positive Sample Values = OD < 1.133

Quality Control:
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

References:

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SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

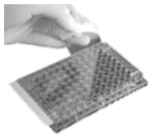
→ Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

3.  Pipette **25 ul Positive / Negative Controls** / **Samples** to the respective wells.

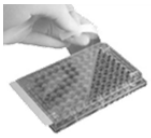
4.  Pipette **50 ul Biotinylated Atezolizumab** to all wells.

4. Cover plate  and incubate for 

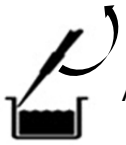
at Room Temperature.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

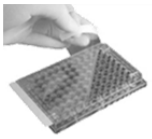
6.  Pipette **50 ul Streptavidin: HRP** into each well.

7. Cover plate  and incubate for 

at Room Temperature.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for 

at Room Temperature.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

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