KRIBIOLISA® Gatipotuzumab ELISA

REF : KBI1354

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Gatipotuzumab in human serum and plasma

RUO	For Research Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biologi isk
	Expiry D		Consu rating Instructions

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REF KBI1354 Σ 96 tests

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Introduction:

Gatipotuzumab is a humanized, glyco-optimized monoclonal antibody engineered to selectively recognize the tumor-associated glycoform of mucin-1 (TA-MUC1), a cancer-specific epitope that is overexpressed on the surface of many epithelial malignancies. Unlike normal MUC1, TA-MUC1 arises from aberrant glycosylation patterns found predominantly on tumor cells, enabling gatipotuzumab to distinguish malignant tissue from healthy cells with high specificity. By binding to TA-MUC1, gatipotuzumab facilitates immune-mediated mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) against targeted cancer cells, making it a promising agent in the field of targeted cancer immunotherapy. Gatipotuzumab has been evaluated in several preclinical and clinical studies across various TA-MUC1 positive cancers, including ovarian, breast, and lung cancers; however, it remains investigational and has not yet received regulatory approval for routine clinical use in major markets such as the United States or the European Union.

Intended Use:

The KRIBIOLISA® Gatipotuzumab ELISA is intended for use as an analytical tool for the quantitative determination of Gatipotuzumab in human serum and plasma.

Principle:

The method employs an indirect enzyme-linked immunosorbent assay (ELISA) technique. MUC1(126-149-Cys)-Tn (131,144) antigen is pre-coated onto microwells. Samples or standards are added to the microwells, and Gatipotuzumab present in the samples binds specifically to the immobilized antigen. After incubation, unbound components are removed by washing, and an HRP (horseradish peroxidase)-conjugated with Anti-human Kappa antibody is added, which binds to the captured Gatipotuzumab. Following a second washing step to remove any unbound conjugate, the ready-to-use substrate 3,3',5,5'-Tetramethylbenzidine (TMB) is added, producing a color reaction proportional to the amount of Gatipotuzumab in the sample. The reaction is stopped with a stop solution, and absorbance is measured at 450 nm to determine the concentration of Gatipotuzumab.

Materials Provided:

Part	Description	Qty
MUC1(126-149-Cys)-Tn (131,144) Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with MUC1(126-149-Cys)-Tn (131,144) antigen.	1 x 96 wells
Gatipotuzumab Standard	Gatipotuzumab Standard in a buffered protein base with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane (lyophilized, concentrated, 30 ug/ml)	2 vials
Anti-human Kappa antibody:HRP Conjugate concentrated	Anti-human Kappa antibody:HRP conjugated to Horseradish Peroxidase, concentrated.	1 vial
Detection Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	50 ml
(1X) Standard Diluent	Buffered protein base with 1:100 dilution human serum and preservatives 0.02% Methylisothiazolinone and 0.02% bromonitrodioxane.	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen.	12 ml
Stop Solution	0.73M Phosphoric Acid.	12 ml
Instruction Manual		1 no

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. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and store the Anti-human Kappa antibody:HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess .Working Anti-human Kappa antibody: HRP Conjugate after running your assay.
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. 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 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.samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation - Serum samples have to be diluted 1:100 (v/v), e.g. for 1:100 (1 ul sample + 99 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. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.
4. **Standards Preparation**: Reconstitute the concentrated lyophilized Standard vial with 250 ul of Standard Diluent to obtain a concentration of 120 ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Prepare **Standards** as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
30 ug/ml	Standard	Lyophilized Standard provided in the Kit
120 ug/ml	Standard No.7	Reconstituted in 250 ul of Standard Diluent (1X
60 ug/ml	Standard No.6	125 ul Standard No.7 + 125 ul Standard Diluent (

5. **Working Anti-human Kappa antibody:HRP Conjugate – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

30 ug/ml	Standard No.5	125 ul Standard No.6 + 125 ul Standard Diluent (
15 ug/ml	Standard No.4	125 ul Standard No.5 + 125 ul Standard Diluent (
7.5 ug/ml	Standard No.3	125 ul Standard No.4 + 125 ul Standard Diluent (
3.75 ug/ml	Standard No.2	125 ul Standard No.3 + 125 ul Standard Diluent (
1.875 ug/ml	Standard No.1	125 ul Standard No.2 + 125 ul Standard Diluent (
0 ug/ml	Standard No.0	Only Standard Diluent (1X)

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Gatipotuzumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Gatipotuzumab present in the sample.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Gatipotuzumab.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. All steps must be performed at 37ºC.

2. Pipette **100 ul** of **prepared Standards and diluted Samples** to the respective wells.

3. Cover the plate and incubate for 60 minutes at 37ºC.

4. 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.

5. Pipette **100 ul** of **Working Anti-human Kappa antibody:HRP Conjugate to** all wells.

6. Cover the plate and incubate for 120 minutes at 37ºC.

7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** as mentioned in Step 4.

8. Add **100 ul** of **TMB Substrate** in each well.

9. Incubate the plate at 37ºC 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.

10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate reader.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown

Gatipotuzumab, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Gatipotuzumab Concentration. If samples were diluted, multiply by the appropriate dilution factor.

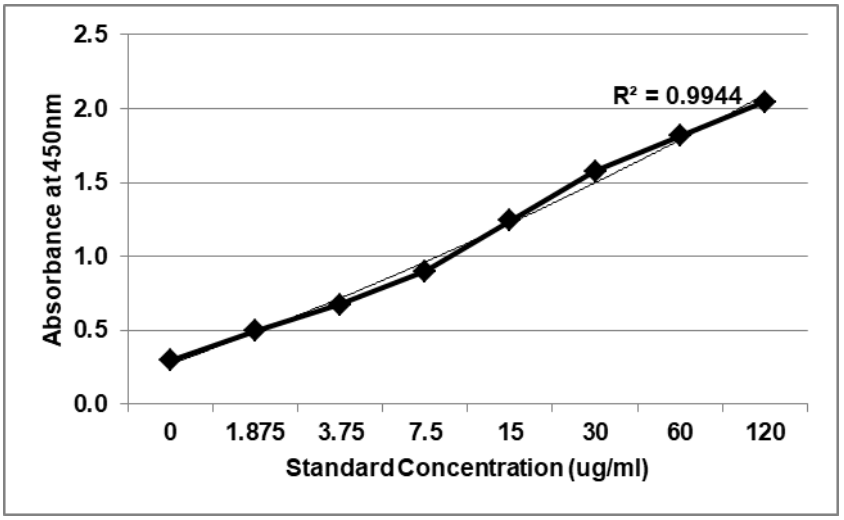
Software which is able to generate a linear regression is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 120 ug/ml standard.

Typical Data (representative only)

Standards (ug/ml)	Absorbance	Interpolated Concentration	% Recovery
0	0.298		
1.875	0.495	1.8	96.7
3.75	0.672	3.8	102.6
7.5	0.901	7.3	97.2
15	1.240	15.3	101.7
30	1.577	30.9	103.0
60	1.814	55.5	92.6
120	2.046	127.5	106.2

Typical Graph (representative only)



Abs = absorbance at 450nm

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.

Performance Characteristics of the Kit:
This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:
Limit of Quantification: It is defined as the lowest concentration of an analyte that can be determined with an acceptable repeatability and the LOQ was found to be 1.8 ug/ml.
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD.

10 replicates of '0' standards were evaluated and the LOD was found to be less than 1.7 ug/ml.

Specificity:
The capture protein used in the kit is a MUC1 (MUC1) antigen, expressed in mammalian cells. The standard used is a Gatipotuzumab biosimilar-grade reagent, also expressed in mammalian cells, exhibiting high-affinity and specific binding to MUC1. This ensures selective detection of Gatipotuzumab without cross-reactivity to unrelated human immunoglobulins, other therapeutic monoclonal antibodies.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (1.875 ug/ml), medium (30 ug/ml) and high (120 ug/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV
Low	<12%
Medium	<10%
High	<10%

Pool	Inter Assay %CV
Low	<15%
Medium	<10%
High	<10%

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.
- 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

SCHEMATIC ASSAY PROCEDURE

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

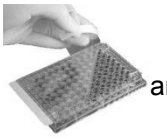
Components (2-8ºC) → 30 mins RT Before Use → 

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

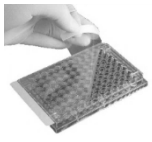
3.  Pipette **100 ul prepared Standards** / **diluted Samples** to the respective wells.

4. Cover plate  and incubate for 

at 37ºC.

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

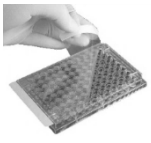
6.  Pipette **100 ul Working Anti-human Kappa antibody:HRP Conjugate** into each well.

7. Cover plate  and incubate for 

at 37ºC.

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 37ºC.



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

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

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KEY

	MTP -149-Cys)-Tn (131,144) Coated Microtiter P 8 wells)
	R STD ant Gatipotuzumab Standard, Lyophilized
	HRP CONJ Kappa antibody:HRP Conjugate d
	DETN DIL luent
	1X SAMP DIL luent
	1X STD DIL Diluent
	20X WASH BUF ffer
	SUB TMB te
	SOLN STOP
	Consult Instructions for Use
	C REF umber FE
	Expiration Date
	Storage Temperature

SYMBOLS