KRIBIOLISA™ Teclistamab (TECVAYLI) ELISA

REF : KBI1667

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Determination of Teclistamab in 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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REF KBI1667 Σ 96 tests

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Introduction:

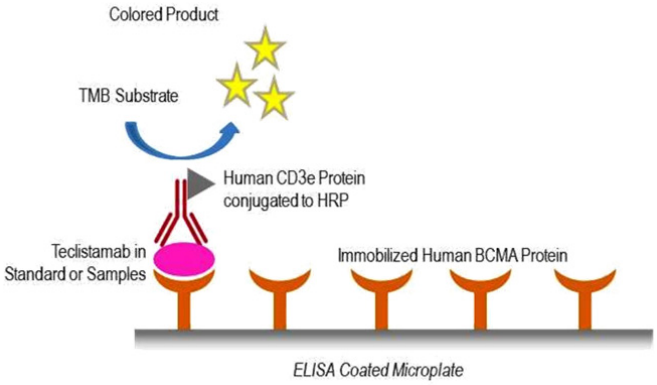
Teclistamab is a bispecific T-cell engager antibody that targets both B-cell maturation antigen (BCMA) expressed on the surface of myeloma cells and the CD3 receptor expressed on the surface of T cells. The FDA in Oct 2022 granted approval to Janssen Biotech's Teclistamab (TECVAYLI) for treatment of relapsed or refractory multiple myeloma, marking a new class of therapy now available for patients whose disease had progressed on other types of therapy.

Intended Use:

The KRIBIOLISA™ Teclistamab ELISA is used as an analytical tool for quantitative determination of Teclistamab in human serum and plasma.

Principle:

The method employs indirect sandwich ELISA technique. Recombinant BCMA protein is pre-coated onto microwells. Samples and Teclistamab standards are pipetted into microwells and bound by the capture protein. After incubation the wells are washed and followed by addition of HRP-conjugated CD3 protein into each well and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Teclistamab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

Part	Description	Qty
BCMA protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with BCMA protein.	1 x 96 wells
Teclistamab Standard	Recombinant Teclistamab in a buffered protein base with preservative sodium azide (lyophilized, 2 ug/ml)	2 vials
CD3 epsilon protein:HRP Conjugate	CD3 epsilon protein:HRP Conjugate with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with 1:1000 dilution human serum and preservative sodium azide < 0.01%	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	2N Sulfuric 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. 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.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

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 1:1000 (v/v), e.g. 1 ul sample + 999 ul sample diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -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 Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 2 ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. 2000 ng/ml is the top standard. Prepare further **Standards** by serially diluting the Top Standard as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
2000 ng/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit + 1ml of Standard Diluent
1000 ng/ml	Standard No.6	500 ul Reconstituted Standard (2 ug/ml) + 500 ul Standard Diluent
500 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
250 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
125 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
62.5 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
31.25 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	Only Standard Diluent

Use the Standards immediately upon reconstitution. Discard balance standard after use. Do not store them for further experiments.

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 Teclistamab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Teclistamab 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 Teclistamab concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
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 Teclistamab.
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. A standard curve is required for each assay. All steps must be performed at 37°C

2. Pipette **100 ul** of prepared **Standards** or diluted **Samples** into the respective wells.

3. Cover the plate and incubate for 90 minutes at 37°C

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. Add **100 ul** of **CD3 epsilon protein:HRP Conjugate** into each well.

6. Cover the plate and incubate for 90 minutes at 37°C

7. 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.

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

9. Incubate the plate at 37°C 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.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log 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 Teclistamab concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve.

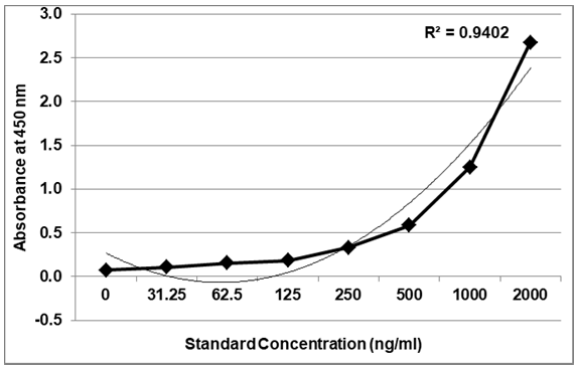
At the point of intersection, draw a vertical line to the X-axis and read the Teclistamab Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4PL (2nd order) is best recommended for automated results.

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 2000 ng/ml standard.

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.072	--	--
31.25	0.107	31.1	99.4
62.5	0.152	74.8	119.7
125	0.183	118.4	94.7
250	0.330	262.3	104.9
500	0.586	486.3	97.3
1000	1.248	1003.8	100.4
2000	2.678	1999.8	100.0

Typical Graph



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 and the Assay Guidance Manual.

Sensitivity:
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 15.6 ng/ml

Specificity:
The capture protein used in the kit is a recombinant BCMA protein expressed using a protein construct made of a DNA sequence encoding the human BCMA (NP_001183.2) (Met1-Ala54). To ensure a high degree of specificity for the assay which detects a bispecific antibody (Teclistamab), the detection protein is a HRP conjugated to recombinant CD3e expressed using a protein construct made of a DNA sequence encoding the human CD3E (NP_000724.1) (Met1-Asp126). The standard used in the kit is a recombinant Teclistamab with IgG4-lambda as receptor identification.

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 (31.25 ng/ml), medium (250 ng/ml) and high (2000 ng/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	Inter Assay %CV
Low	<12%	<12%
Medium	<12%	<12%
High	<10%	<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.
- 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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Teclistamab induction therapy for Crohn disease previously treated with infliximab: a randomized trial ... WJ Sandborn, P Rutgeerts, R Enns… - Annals of internal …, 2007 - Am Coll Physicians

Teclistamab therapy for moderate to severe psoriasis: a randomized, controlled phase III trial ... A Menter, SK Tyring, K Gordon, AB Kimball… - Journal of the American …, 2008 - Elsevier

SCHEMATIC ASSAY PROCEDURE

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

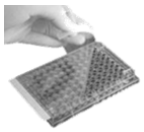
Components (2-8ºC) → 30 mins RT Before Use → 

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

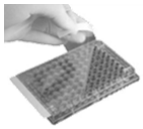
3.  Pipette **100 ul** prepared **Standards** / diluted **Samples** into each well.

4. **Cover plate**  **and incubate for** 

at 37°C.

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

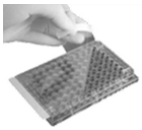
6.  Pipette **100 ul CD3e:HRP** into each well.

7. Cover plate  and incubate for 

at 37°C.

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 37°C.

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

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

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Teclistamab equivalent
1A 2A	zero std zero std			
1B 2B	31.25 ng/ml 31.25 ng/ml			
1C 2C	62.5 ng/ml 62.5 ng/ml			
1D 2D	125 ng/ml 125 ng/ml			
1E 2E	250 ng/ml 250 ng/ml			
1F 2F	500 ng/ml 500 ng/ml			
1G 2G	1000 ng/ml 1000 ng/ml			
1H 2H	2000 ng/ml 2000 ng/ml			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	**BCMA protein Coated Microtiter Plate (12x8 wells)**
STD	**Anti-Teclistamab Standard, lyophilized**
HRP CONJ	**Conjugate Horseradish Peroxidase**
1X STD DIL	**(1X) Standard Diluent**
1X SAMP DIL	**(1X) Sample Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**