KRIBIOLISA™ Daratumumab (Darzalex™) ELISA

REF : KBI1028

Ver 4.2

RUO

Enzyme Immunoassay for the quantitative determination of Daratumumab in serum, plasma and cell culture supernatant

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

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KBI1028 **96 tests** **REF** 

KRISHGEN BioSystems| For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
For Asia / India: tel: +91(22) - 49198700
Email: sales@krishgen.com

Introduction:

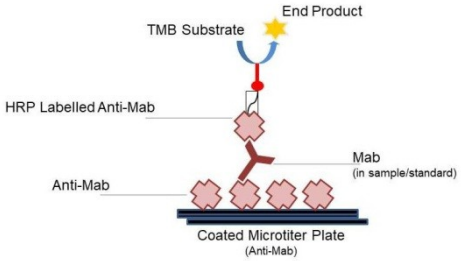
Daratumumab (trade name Darzalex) is an anti-cancer drug. It binds to CD38, which multiple myeloma cells overexpress. Daratumumab was originally developed by Genmab, but it is now being jointly developed by Genmab along with the Johnson & Johnson subsidiary Janssen Biotech, which acquired worldwide commercialization rights to the drug from Genmab.Daratumumab was given breakthrough therapy drug status in 2013 for multiple myeloma. It was awarded orphan drug status for multiple myeloma, diffuse large B cell lymphoma, follicular lymphoma, and mantle cell lymphoma.

Intended Use:

The KRIBIOLISA™ Daratumumab (Darzalex™) ELISA is used as an analytical tool for quantitative determination of Daratumumab in serum, plasma and cell culture supernatant.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Daratumumab are pre-coated onto microwells. HRP Conjugate, Samples / Standards are pipetted into microwells and human Daratumumab present in the sample are bound by the capture antibody. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Daratumumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

Part	Description	Qty
Anti-Daratumumab Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Daratumumab monoclonal antibody.	1 x 96 wells
Daratumumab Standard	Recombinant Daratumumab in a buffered protein base with preservative sodium azide– lyophilized (3 ug/ml)	2 vials
Anti-Daratumumab:HRP Conjugate	Anti-Daratumumab conjugated to Horseradish Peroxidase 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%	50 ml
(1X) Standard Diluent	Buffered protein base with 1:100 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	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 25µl to 1000µl
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.

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 1:10 or 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 -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 (1X) to obtain a concentration of 3ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 853.4 ul of reconstituted **Standard (3 ug/ml)** with 146.6 ul of Standard Diluent (1X) to generate a **2560 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent (1X) as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
3 ug/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit + 1ml of Standard Diluent (1X)
2560 ng/ml	Standard No.6	853.4 ul Reconstituted Standard (3 ug/ml) + 146.6 ul Standard Diluent (1X)
1280 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent (1X)
640 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent (1X)
320 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent (1X)
160 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent (1X)
80 ng/ml	Standard No.1	500 ul Standard No.2+ 500 ul Standard Diluent (1X)
0 ng/ml	Standard No.0	Only Standard Diluent (1X)

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 Daratumumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Daratumumab 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 Daratumumab 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 Daratumumab.
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 Controls 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 out **100 ul** of **Standards** or **Samples** in each well and incubate at 37°C for 60 minutes.

3. 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.

4. Add **100 ul** of **Anti-Daratumumab:HRP detection conjugate** into the respective wells and incubate at 37°C for 60 minutes.

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

7. 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.

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

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

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate 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 Daratumumab 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 Daratumumab Concentration.

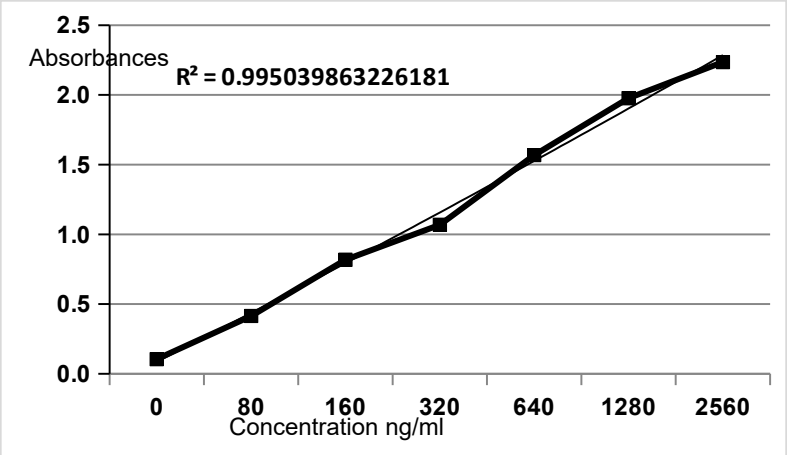
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

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 2560 ng/ml standard.

Typical Data

Standard (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.105	--	--
80	0.415	68.8	86.0
160	0.818	188.2	117.7
320	1.069	292.7	91.5
640	1.568	643.9	100.6
1280	1.976	1326.1	103.6
2560	2.235	2496.0	97.5

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.

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 less than 80 ng/ml

Specificity:
The antibodies used in the kit are monoclonal antibodies, anti-idiotypic and specific for Daratumumab. The calibrators/ standards used are calibrated against commercially sourced (Darzalex™).

Linearity:

Standards provided in the kit will be used for measuring the linearity range of Daratumumab present in matrix. Serum and Plasma (sodium heparin as anti-coagulant) were used to measure dilutional linearity and recovery with matrix effect (n=2).

Serum sample diluted 1:10 with Sample Diluent

Standard (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.152	--	--
80	0.188	150.5	188.1
160	0.174	93.3	58.3
320	0.241	337.3	105.4
640	0.335	637.8	99.7
1280	0.531	1280.5	100.0
2560	0.836	2559.9	100.0

Serum sample Diluted 1:100 with Sample Diluent

Standard (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.105	1.0	--
80	0.415	68.8	86.0
160	0.818	188.2	117.7
320	1.069	292.7	91.5
640	1.568	643.9	100.6
1280	1.976	1326.1	103.6
2560	2.235	2496.0	97.5

Plasma (Sodium Heparin) sample diluted 1:500 and 1:1000 respectively with Sample Diluent

Standard (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.081	--	--
2560	2.486	2729.3	106.6
0	0.081	--	--
2560	2.528	2980.3	116.4

Standards serially diluted with Sample Diluent spiked in serum

Standard (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.081	--	--
5	0.136	6.0	120.0
15	0.168	13.7	91.7
75	0.501	89.5	119.3

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 (80 ng/ml), medium (320 ng/ml) and high (2560 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	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

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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Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma
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Direct in Vitro Comparison of Daratumumab with Surrogate Analogs of CD38 Antibodies MOR03087, SAR650984 and Ab79
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Daratumumab for the treatment of multiple myeloma…Touzeau C, Moreau P… Expert Opin Biol Ther….2017…Taylor & Francis

SCHEMATIC ASSAY PROCEDURE

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

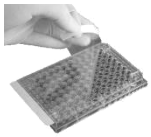
Components (2-8ºC) → 30 mins RT Before Use → 

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

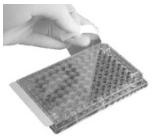
3.  Pipette **100 µl Standards** / **Samples** into each well.

4. Cover plate  and incubate for 

at 37°C.

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

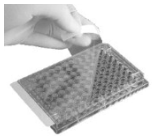
6.  Pipette **100 µl Anti Daratumumab:HRP** into each well.

7. Cover plate  and incubate for 

at 37°C.

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

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

10. Cover plate  and incubate for 

at 37°C.

11.  Pipette **100 µl 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 Daratumumab equivalent
1A 2A	zero std zero std			
1B 2B	80 ng/ml 80 ng/ml			
1C 2C	160 ng/ml 160 ng/ml			
1D 2D	320 ng/ml 320 ng/ml			
1E 2E	640 ng/ml 640 ng/ml			
1F 2F	1280 ng/ml 1280 ng/ml			
1G 2G	2560 ng/ml 2560 ng/ml			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

THANK YOU FOR USING KRISHGEN PRODUCT !

SYMBOLS KEY

	Anti-Daratumumab Coated Microtiter Plate (12X8 wells)
	Daratumumab Standard, lyophilized
	Conjugate Horseradish Peroxidase
	(1X) Standard Diluent
	(1X) Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalogue Number
	Expiration Date
	Storage Temperature