KRIBIOLISA™ Dasatinib (SPRYCEL) ELISA

REF : KOD1016

Ver1.1

RUO

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

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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Introduction:

Dasatinib, sold under the brand name Sprycel, is a targeted therapy medication used to treat certain cases of chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL). Specifically it is used to treat cases that are Philadelphia chromosome-positive (Ph+). It is a tyrosine-kinase inhibitor and works by blocking a number of tyrosine kinases such as Bcr-Abl and the Src kinase family.

Intended Use:

The KRIBIOLISA™ Dasatinib ELISA kit is used for quantitative estimation of Dasatinib in human serum and plasma. The kit is for research use only and not for diagnostic use.

Principle:

The Dasatinib ELISA is a competitive inhibition immunoassay for the determination of Dasatinib. A varying concentration of unlabeled Dasatinib Standard or diluted Sample, constant concentration of ABL1 protein and biotin conjugated Anti-ABL1 are added and incubated to the microtitre plate coated with Anti-ABL1. Dasatinib inhibits the binding of Anti-ABL1 to ABL1 protein. Streptavidin:HRP conjugate is added and incubated. Upon washing, unbound Strep:HRP Conjugate will be removed. Bound Streptavidin:HRP conjugate complex will produce a soluble blue colored product after the addition of TMB Substrate. The enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is inversely proportional to the amount of bound Dasatinib present in the standards or samples.

Materials Provided:

Part	Description	Qty
Human Tyrosine kinase (ABL1) monoclonal antibody coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human Tyrosine kinase (ABL1) monoclonal antibody.	1 x 96 wells
Human ABL1 Protein	Recombinant Human ABL1 (concentrated, 100 ug/ml, 10 ul)	1 vial
Strep:HRP Conjugate	Streptavidin HRP Conjugate reagent solution	6 ml
Biotin - ABL1 Antibody	Biotinylated ABL1 antibody solution	1 ml
(5X) ABL1 Protein Diluent	Buffered protein base and preservative sodium azide < 0.01%	10 ml
Dasatinib Standard	Dasatinib Standard (concentrated, 100 ug/ml, 10 ul) in buffered protein base and <0.05% DMSO v/v	1 vial
(1X) Dasatinib Standard Diluent	Buffered protein base with 1:1000 dilution human serum and preservative sodium azide < 0.01%	10 ml
(1X) Sample Diluent	Buffered protein base and preservative sodium azide < 0.01%	2 x 50 ml
(30X) Wash Buffer	30-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	20 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	6 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.
9. Incubator

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:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.
Note: *Grossly hemolyzed samples are not suitable for use in this assay*

Preparation Before Use:

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

Samples should be diluted 1:1000 (v/v) for optimal recovery, (for example 1 ul sample + 999 ul Sample Diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent accordingly.

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 (1X) Wash Buffer; dilute 20 ml of (30X) Wash Buffer in 580 ml of DI water.
4. To make (1X) ABL1 Protein Diluent, dilute 1 ml of (5X) ABL1 Protein Diluent with 4 ml of DI water.
5. **ABL1 Protein Preparation** – Add 1 ul of concentrated ABL1 Protein (100 ug/ml) with 99 ul (1X) ABL1 protein diluent to get 1 ug/ml ABL1 mid stock. Then add 2 ul of 1 ug/ml ABL1 mid stock to 998 ul of (1X) ABL1 Protein diluent to get ABL1 Protein working solution.
6. Dasatinib Standard preparation – Add 1 ul of concentrated Dasatinib with 99 ul of Dasatinib Standard Diluent to get 1 ug/ml Dasatinib mid stock. Please refer to the table below for further dilutions.

Standard Conc.	Standard No.	Dilution Particulars
100 ug/ml	Main stock	Original Standard
1 ug/ml	Mid stock	1 ul Original Standard + 99 ul Dasatinib Standard Diluent (1X)
12,000 pg/ml	Standard No. 6	12 ul of Mid Stock + 988ul Dasatinib Standard Diluent (1X)
10,000 pg/ml	Standard No. 5	10 ul of Mid Stock + 990ul Dasatinib Standard Diluent (1X)
8000 pg/ml	Standard No.4	8 ul of Mid Stock + 992ul Dasatinib Standard Diluent (1X)
4000 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Dasatinib Standard Diluent (1X)
500 pg/ml	Standard No.2	125 ul Standard No.3 + 875 ul Dasatinib Standard Diluent (1X)
0 pg/ml	Standard No.1	250 ul Dasatinib Standard Diluent (1X)

Mix each tube thoroughly before the next transfer.

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. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of Dasatinib.
3. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. 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.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.
8. Making serial dilution in the wells directly is not permitted.
9. Prepare the Standard within 15 minutes prior to running the assay.
10. Please carefully dilute Standards according to the instruction, and avoid foaming. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettes are calibrated.
11. If crystals have formed in the Wash Solution (30X) concentrate, warm to room temperature and mix gently until the crystals are completely dissolved.
12. Contaminated water or container for reagent preparation will influence the detection results.

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.

2. Add **50 ul ABL1 Protein working solution** to respective wells.

3. Pipette **10 ul Biotinylated ABL1 Antibody** solution to all wells.

4. **Add 50 ul of Dasatinib Standard / diluted Samples** to all wells and mix properly**.**

5. Cover the plate with a sealer and incubate for **60 minutes** at **37°C.**

6. Pipette **50 ul Streptavidin:HRP Conjugate** to all wells. Mix well.

7. Cover the plate with a sealer and incubate for **60 minutes** at **37°C.**

8. Aspirate and wash plate 5 times with diluted **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. Pipette **100 ul TMB Substrate** in all the wells.

10. Incubate the plate at **37°C** for **30 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

11. Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

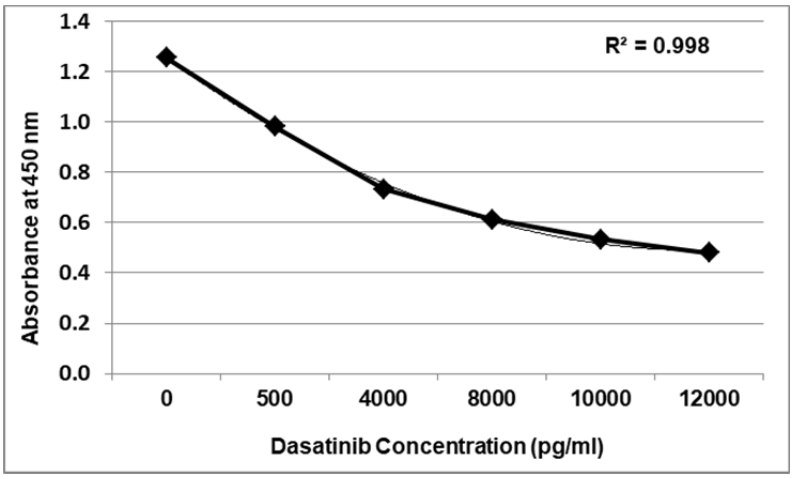
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the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

Typical Data

Dasatinib Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	1.255	--	--
500	0.981	561.1	112.2
4000	0.734	3862.9	96.6
8000	0.612	7255.2	90.7
10000	0.534	10221.9	102.2
12000	0.480	12689.0	105.7

Typical Graph



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.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	pg/ml Dasatinib equivalent
1A 2A	zero std zero std			
1B 2B	500 pg/ml 500 pg/ml			
1C 2C	4000 pg/ml 4000 pg/ml			
1D 2D	8000 pg/ml 8000 pg/ml			
1E 2E	10,000 pg/ml 10,000 pg/ml			
1F 2F	12,000 pg/ml 12,000 pg/ml			
1G 2G	Sample			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

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