KRIBIOLISA™ Ivacaftor (KALYDECO®) ELISA

REF: KOD1017

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Determination of Ivacaftor 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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REF **KOD1017** Σ **96 tests**

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:

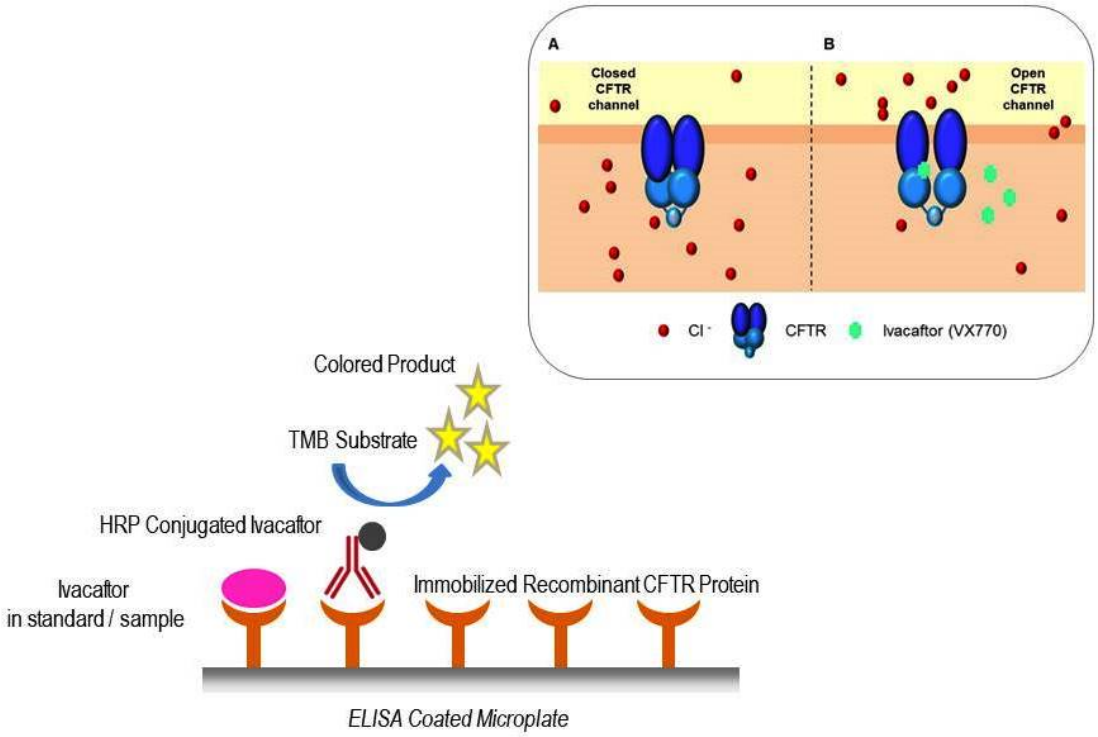
Ivacaftor is a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator used alone or in combination products to treat cystic fibrosis in patients who have specific genetic mutations that are responsive to the medication. Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF). It is manufactured and distributed by Vertex Pharmaceuticals. It was approved by the Food and Drug Administration on January 31, 2012. Ivacaftor is administered as a monotherapy and also administered in combination with other drugs for the management of CF.

Intended Use:

The KRIBIOLISA™ Ivacaftor (KALYDECO®) ELISA is used as an analytical tool for quantitative determination of Ivacaftor in human serum and plasma.

Principle:

The method employs the competitive enzyme immunoassay technique. In the first step, samples and standards along with the Ivacaftor: HRP conjugate is pipetted and incubated. Both the standard/sample and Ivacaftor: HRP conjugate compete with CFTR (cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7), which is pre-coated onto microwells. Free HRP conjugate will be removed by a washing step. In the second step, TMB substrate is added to microwells and color develops inversely proportionally to the amount of Ivacaftor present in the samples or standards. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



PRINCIPLE OF THE KRIBIOLISA™ IVACAFTOR ELISA

Materials Provided:

Part	Description	Qty
CFTR Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant human CFTR.	1 x 96 wells
Recombinant Ivacaftor Standard	Recombinant Ivacaftor Standard (concentrated. 1 mg/ml)	1 vial
(1X) Standard Diluent	Buffered protein base with 1:1000 human serum and with preservative thiomersol <0.01%	10 ml
Ivacaftor:HRP Conjugate	Ivacaftor 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%	2 x 50 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. 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.

Storage Information:

1. Store main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. After reconstitution of standards, it has to be used immediately and cannot be stored.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

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

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 in 1000 (v/v), e.g. 1 ul sample in 999 ul of (1X) Sample diluent, prior to assay. The samples may be kept at 2 -8ºC for up to three days. Long-term storage requires -20ºC.

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 Insulin Glargine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Insulin Glargine 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 Insulin Glargine 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 (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of Insulin Glargine.
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.

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. The Main standard Concentration is as per accompanying Reagent Sheet available in the kit. Prepare standards as per instructions and use as indicated.

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 Insulin Glargine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Insulin Glargine 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 Insulin Glargine.
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. Add 100 ul of **prepared Standards/Samples** into their respective well.

2. Add 100 ul of **Ivacaftor:HRP Conjugate** into each well.

3. Incubate at 37°C for 90 minutes.

4. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.

5. Add 100 ul per well of **TMB Substrate**. Cover the plate

6. Incubate at Room Temperature under dark for 30 minutes.

7. Add 100 ul of **Stop Solution** to each microwell.

8. Measure the optical density of the wells on a plate reader at 450 nm within 10 minutes.

Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown Ivacaftor concentrations, find the unknowns 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 Ivacaftor concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software, 4-PL or cubic spline or polynomial regression (2^{nd} order) may be preferred.

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.

Specificity:
The recombinant protein used in the kit is generated for CFTR. The standard / calibrator used in the kit are calibrated against commercially sourced Kalydeco Injection™.

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:

In January 2012, ivacaftor (Kalydeco; Vertex Pharmaceuticals), a small-molecule potentiator of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel, was approved by the US Food and Drug Administration (FDA) for the treatment of patients with …
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