

KRIBIOLISA® Pertuzumab (PERJETA™) ELISA

REF : KBI1086

Ver 2.1

RUO

Enzyme Immunoassay for the Quantitative Determination of Pertuzumab
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

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Krishgen Biosystems Private Limited is strictly prohibited.

REF KBI1086 **96 tests****Krishgen Biosystems Private Limited**

For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005

For Asia/India Customers: tel +91(22)-49198700

Email: sales1@krishgen.com | <http://www.krishgen.biz> / www.krishgenbio.com

Introduction:

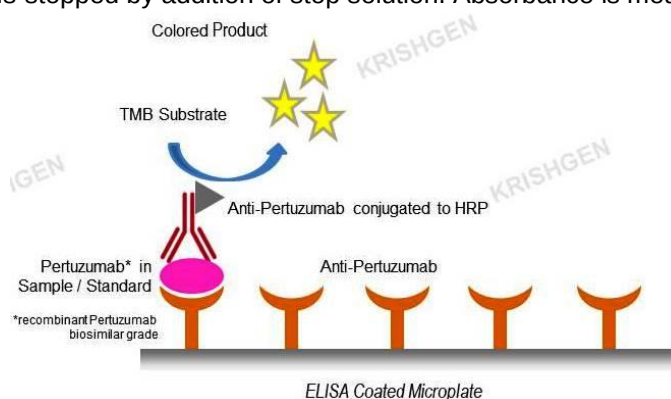
Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (subdomain II) of the human epidermal growth factor receptor 2 protein (HER2). It consists of two heavy chains and two light chains that have 448 and 214 residues respectively. It was first approved by the FDA in 2012 for use with docetaxel and another HER2-targeted monoclonal antibody, trastuzumab, in the treatment of metastatic HER2-positive breast cancer. Its indicated conditions have since expanded to include use as both a neoadjuvant therapy and an adjuvant therapy in the treatment of HER2-positive breast cancers at high risk of recurrence.

Intended Use:

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

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Pertuzumab are pre-coated onto microwells. Samples and standards are pipetted into microwells and human Pertuzumab present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Pertuzumab antibody is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use 3,3',5,5' Tetra Methyl Benzidine substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Pertuzumab in the sample. Color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

Part	Description	Qty
Anti-Pertuzumab Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Pertuzumab monoclonal antibody	1 x 96 wells
Pertuzumab Standard	Recombinant Pertuzumab in a Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane- (lyophilized, concentrated 3 ug/ml)	2 vials
Anti-Pertuzumab:HRP Conjugate Concentrated	Anti-Pertuzumab conjugated to HRP concentrated (0.5 mg/ml)	2 vials
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% methylisothiazolinone and 0.02% bromonitrodioxane	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane with 1:1000 dilution of human serum	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersal < 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. Standard graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. It is advisable to aliquot and stores the Anti-Pertuzumab: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-Pertuzumab: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 (18-25°C). 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.

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 - Serum and Plasma samples have to be diluted **1:1000 (v/v)**, e.g. for 1:1000 (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 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 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 853.3 ul of reconstituted **Standard (3 ug/ml)** with 146.7 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	Reconstituted Standard	Lyophilized Standard provided in the Kit + 1 ml of Standard Diluent (1X)
2560 ng/ml	Standard No.7	853.3 ul Reconstituted Standard + 146.7 ul Standard Diluent (1X)
1280 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent (1X)

Standard Concentration	Standard Vial	Dilution Particulars
640 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent (1X)
320 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent (1X)
160 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent (1X)
80 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent (1X)
40 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)

Mix each tube thoroughly before the next transfer. Use the standards for experiment within one hour of preparation of standard. Discard standard after use.

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

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 Pertuzumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Pertuzumab 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 Pertuzumab.
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 compromise 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. Add **100 ul** of **prepared Standards or diluted Samples** into the respective wells.
3. Cover the plate and incubate for 60 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 off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
5. Add **100 ul** of **Working Anti-Pertuzumab:HRP Conjugate** into each well.
6. Cover the plate and incubate for 60 minutes at 37°C.
7. Aspirate and wash plate 4 times with **Wash Buffer (1X)** same as in step 4.
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 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 Standard graph paper, plot the average value (absorbance 450 nm) 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 Pertuzumab 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 Pertuzumab 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:

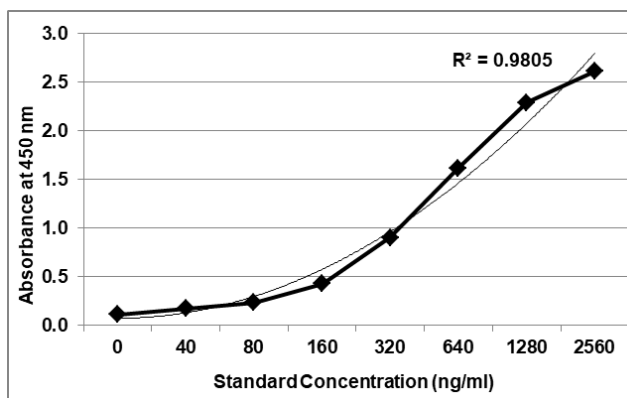
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 2560 ng/ml standard.

Typical Data (representative only)

Standard Concentration (ng/ml)	Absorbance A	Absorbance B	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.093	0.135	0.114	--	--
40	0.157	0.198	0.177	48.9	122.2
80	0.265	0.214	0.239	81.9	102.4
160	0.427	0.438	0.432	158.7	99.2
320	0.928	0.885	0.907	321.8	100.5
640	1.642	1.597	1.619	633.0	98.9
1280	2.306	2.279	2.293	1308.4	102.2
2560	2.582	2.642	2.612	2498.2	97.6

Typical Graph (representative only)



Quality Control:

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 38.50 ng/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 35.50 ng/ml.

Specificity:

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

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 (40 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/v) 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:**

Improving Pertuzumab production by gene optimization and proper signal peptide selection
 A Ramezani, EM Maymand... - Protein expression and ..., 2017 - Elsevier

Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex
 MC Franklin, KD Carey, FF Vajdos, DJ Leahy... - Cancer cell, 2004 - Elsevier

Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer
 DB Agus, MS Gordon, C Taylor, RB Natale... - Journal of clinical ..., 2005 - nlp.case.edu

The influence of glycans-specific bioconjugation on the FcγRI binding and in vivo performance of 89Zr-DFO-pertuzumab
 D Vivier, K Fung, C Rodriguez, P Adumeau... - Theranostics, 2020 - ncbi.nlm.nih.gov

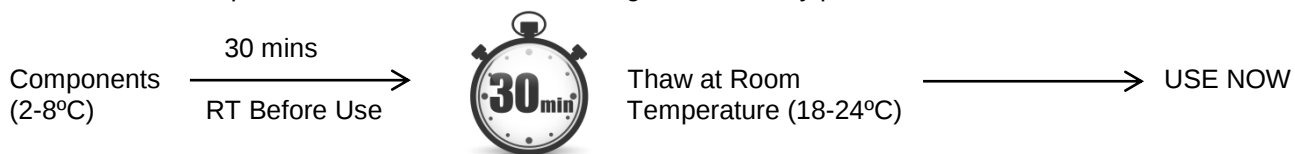
Humanization of a recombinant monoclonal antibody to produce a therapeutic HER dimerization inhibitor, pertuzumab
 CW Adams, DE Allison, K Flagella, L Presta... - Cancer Immunology ..., 2006 - Springer

Evaluating the predictive value of biomarkers for efficacy outcomes in response to pertuzumab-and trastuzumab-based therapy: an exploratory analysis of the ...
 A Schneeweiss, S Chia, R Hegg... - Breast Cancer ..., 2014 - breast-cancer-research ...

Pertuzumab in Combination with Trastuzumab Shows Significantly Enhanced Antitumor Activity in HER2-Positive Human Gastric Cancer Xenograft ...
 Y Yamashita-Kashima, S Iijima, K Yoroza... - Clinical Cancer ..., 2011 - AACR

SCHEMATIC ASSAY PROCEDURE

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



2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



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

4. Cover plate and incubate for **60 min** at 37°C.

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

6. Pipette **100 ul Working Anti-Pertuzumab:HRP** into each well.

7. Cover plate and incubate for **60 min** 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 **30 min** at 37°C.

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

12. Read absorbance at 450 nm with a microplate reader within **15 min** of stopping reaction.

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited 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.

Krishgen Biosystems Private Limited. 2026

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED®, GENLISA®, DHARMAPLEX®, GENBULK®, GENLISA®, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ©KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

PERJETA™ is the registered trademark of Roche Genetech Inc.

SYMBOLS KEY

MTP	Anti-Pertuzumab Coated Microtiter Plate (12x8 wells)
STD	Pertuzumab Standard, Lyophilized
HRP CONJ	Anti-Pertuzumab:HRP Conjugate Concentrated
DETN DIL	Detection Diluent
1X SAMP DIL	(1X) Sample Diluent
1X STD DIL	(1X) Standard 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