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# KRIBIOLISA™ Risankizumab ELISA

**REF** : KBI1593

Ver 1.0

**RUO**

Enzyme Immunoassay for the Quantitative Determination of Risankizumab 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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**KBI1593** **96 tests** **REF** Σ

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**Introduction:**

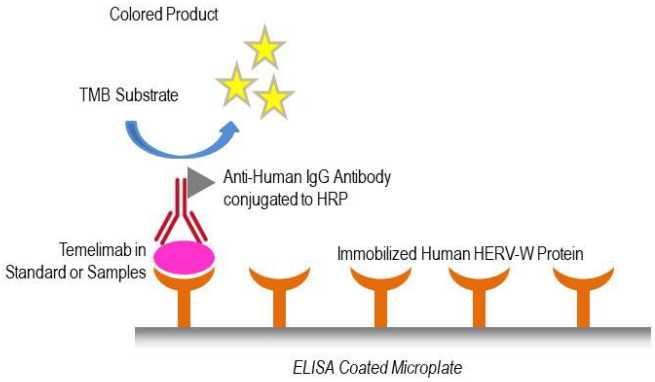
Risankizumab is a humanized immunoglobulin (Ig) G1 monoclonal antibody that specifically targets the p19 subunit of interleukin (IL)-23, thereby inhibiting IL-23-dependent cell signaling. Risankizumab was approved by the U.S. FDA for treatment of moderate-to-severe plaque psoriasis in April 2019, and in vitro, has been more potent in inhibiting IL-23 signalling than other drugs performing in the same IL-23 complex.

**Intended Use:**

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

**Principle:**

IL-23A Protein is pre-coated onto microwells. Samples and standards are pipetted into microwells. Risankizumab present in the sample is bound by the protein. Then, an Anti-Human IgG conjugated to HRP is pipetted and incubated. 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 Risankizumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.




**Materials Provided:**

| Part | Description | Qty |
|---|---|---|
| IL-23A Coated Microtiter Plate | 96 well polystyrene microplate (12 strips of 8 wells) coated with IL-23A protein. | 1 x 96 wells |
| Recombinant Risankizumab Standard | Recombinant Risankizumab in a buffered protein base with preservative sodium azide– lyophilized (1 ug/ml) | 2 vials |
| Anti-Human IgG:HRP Conjugate | Anti-Human IgG: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 1 ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 320 ul of original **Standard (1 ug/ml)** with 180 ul of Standard Diluent to generate a **640 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

| Standard Concentration | Standard Vial | Dilution Particulars |
|---|---|---|
| 1 ug/ml | Lyophilized Standard | Lyophilized Standard provided in the Kit + 1ml of Standard Diluent |
| 640 ng/ml | Standard No.7 | 320 ul Reconstituted Standard (1 ug/ml) + 180 ul Standard Diluent |
| 320 ng/ml | Standard No.6 | 250 ul Standard No.7 + 250 ul Standard Diluent |
| 160 ng/ml | Standard No.5 | 250 ul Standard No.6 + 250 ul Standard Diluent |
| 80 ng/ml | Standard No.4 | 250 ul Standard No.5 + 250 ul Standard Diluent |
| 40 ng/ml | Standard No.3 | 250 ul Standard No.4 + 250 ul Standard Diluent |
| 20 ng/ml | Standard No.2 | 250 ul Standard No.3 + 250 ul Standard Diluent |
| 10 ng/ml | Standard No.1 | 250 ul Standard No.2 + 250 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 Risankizumab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Risankizumab 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 Risankizumab 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 Risankizumab.
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 **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 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 **Anti-Human IgG: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)** 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 Risankizumab 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 Risankizumab 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 640 ng/ml standard.

**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:**

Blair HA. Risankizumab: A Review in Moderate to Severe Plaque Psoriasis. Drugs. 2020 Aug;80(12):1235-1245. doi: 10.1007/s40265-020-01357-1. Erratum in: Drugs. 2020 Sep;80(14):1505. PMID: 32632826; PMCID: PMC7475056.

Pang Y, Khatri A, Suleiman AA, Othman AA. Clinical Pharmacokinetics and Pharmacodynamics of Risankizumab in Psoriasis Patients. Clin Pharmacokinet. 2020 Mar;59(3):311-326. doi: 10.1007/s40262-019-00842-5. PMID: 31758502; PMCID: PMC7051925.

Gordon B, Strober B et al. Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials, The Lancet, Volume 392, Issue 10148, 2018

McKeage, K., Duggan, S. Risankizumab: First Global Approval. *Drugs* **79**, 893–900 (2019).

Papp, Blauvelt et al. Risankizumab versus Ustekinumab for Moderate-to-Severe Plaque Psoriasis. The New England Journal of Medicine (2017)

## SCHEMATIC ASSAY PROCEDURE

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

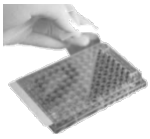
Components (2-8ºC) → 30 mins RT Before Use → 

→ 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

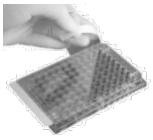
3.  Pipette **100 ul Standards** / diluted **Samples** into each well.

4. Cover plate  and incubate for 

at 37°C.

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

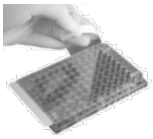
6.  Pipette **100 ul Anti-Human IgG:HRP** into each well.

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 450 nm | Mean Absorbance | ng/ml Risankizumab equivalent |
|---|---|---|---|---|
| 1A<br>2A | zero std<br>zero std | | | |
| 1B<br>2B | 10 ng/ml<br>10 ng/ml | | | |
| 1C<br>2C | 20 ng/ml<br>20 ng/ml | | | |
| 1D<br>2D | 40 ng/ml<br>40 ng/ml | | | |
| 1E<br>2E | 80 ng/ml<br>80 ng/ml | | | |
| 1F<br>2F | 160 ng/ml<br>160 ng/ml | | | |
| 1G<br>2G | 320 ng/ml<br>320 ng/ml | | | |
| 1H<br>2H | 640 ng/ml<br>640 ng/ml | | | |
| 3A<br>4A | Sample | | | |
| 3B<br>4B | Sample | | | |

## LIMITED WARRANTY

Krishgen Biosystems 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, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems 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.

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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 A KRISHGEN PRODUCT!***

## SYMBOLS KEY

| | |
|---|---|
| MTP | IL-23A protein Coated Microtiter Plate (12X8 wells) |
| STD | Risankizumab 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 |