

KRIBIOLISA® Anti-Adalimumab ELISA

REF : KBI2015-QL

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Determination of Anti-Adalimumab 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 KBI2015-QL **96 tests****Krishgen Biosystems Private Limited**

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Introduction:

Adalimumab (trade name Humira) is a fully human monoclonal antibody of IgG1 isotype directed against tumour necrosis factor alpha (TNF- α). It binds both soluble and membrane-bound TNF- α , preventing interaction with the p55 and p75 cell surface receptors and thereby interrupting the downstream inflammatory cascade. It was developed by BASF BioResearch and Abbott Laboratories, now AbbVie. The US FDA approved adalimumab in December 2002 for rheumatoid arthritis and subsequently for psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, plaque psoriasis, hidradenitis suppurativa, uveitis and juvenile idiopathic arthritis. The development of anti-adalimumab antibodies is well documented and is associated with reduced serum drug concentrations and secondary loss of clinical response.

Anti-Drug Antibodies (ADA) may induce unwanted side effects in biopharmaceuticals. Hence, ADA has been subjected to increased scrutiny by the regulatory authorities using immunogenicity safety studies. ADA has been observed in pre-clinical and clinical studies, resulting in significant changes in toxicology, pharmacokinetics and efficacy. These effects result from the generation of drug-induced (neutralizing) autoantibodies against Adalimumab and can be responsible for allergic reaction, or even anaphylactic shock. This ELISA kit detects antibodies for Anti-Adalimumab and may be used for monitoring immunogenicity.

Intended Use:

The KRIBIOLISA® Anti-Adalimumab ELISA is used as an analytical tool for qualitative determination of Anti-Adalimumab in human serum and plasma.

Principle:

The method employs the double antigen bridging (sandwich) enzyme immunoassay technique. Adalimumab is pre-coated onto microwells. Controls or diluted samples are pipetted into the microwells and antibodies to Adalimumab present in the controls or sample bind to the immobilized Adalimumab by one antigen-binding arm. After washing the microwells to remove unbound material, Adalimumab:HRP conjugate is pipetted and incubated; the conjugate binds the remaining free antigen-binding arm of the captured antibody, forming a bridge. Unbound HRP conjugate is removed by a washing cycle. The ready to use substrate solution (TMB) is added to microwells and color develops in direct proportion to the amount of Anti-Adalimumab present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm and the result is reported qualitatively against a cut-off value derived from the Negative Controls.

Materials Provided:

1. Adalimumab Coated Microtiter Plate (12x8 wells) – 1 no
2. Positive Control – 1 vial
3. Negative Control – 1 vial
4. Adalimumab:HRP Conjugate – 10 ml
5. Sample Diluent – 60 ml
6. Wash Buffer (20X) – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

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. Log graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2°C to 8°C 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 (18-25°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 - Samples have to be diluted 1:10 to 1:100 (v/v), e.g. for 1:10 (1 ul sample + 9 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**.

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 Anti-Adalimumab. High Dose Hook Effect arises when excess antibody saturates both the coated Adalimumab and the Adalimumab:HRP conjugate separately, preventing bridge formation and suppressing signal. 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 Anti-Adalimumab 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 Anti-Adalimumab.
4. It is recommended that all Controls 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 Controls and Samples.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at 37°C.
2. Pipette **100 ul** of **Positive Control, Negative Control and diluted Samples** to 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. Pipette **100 ul** of **Adalimumab:HRP Conjugate** to all wells.
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.

Interpretation of the Results:

For the run to be valid, the OD450 nm of Positive Control should be ≥ 1.00 and the OD450 nm of each Negative Control should be <0.200 , if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD450nm of the negative controls by 3 or 3SD (standard deviation)

i.e.

If "Sample OD450/the mean OD450 of Negative Controls" is $\geq$ cut-off, the sample is POSITIVE

If "Sample OD450/the mean OD450 of Negative Controls" is $<$ cut-off the sample is NEGATIVE

Typical Data

Sample	Abs A	Abs B	Mean Abs	Sample OD / Mean NC OD	Result
Negative Control	0.108	0.114	0.111	1.0	--
Positive Control	1.402	1.371	1.387	12.5	POSITIVE
Sample 1	0.126	0.131	0.129	1.2	NEGATIVE
Sample 2	0.515	0.489	0.502	4.5	POSITIVE

Values shown are representative only; a cut-off must be established in each laboratory from the Negative Controls of that run.

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.

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:

Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up...Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW...JAMA...2011...American Medical Association

Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis...Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L...Ann Rheum Dis...2007...BMJ

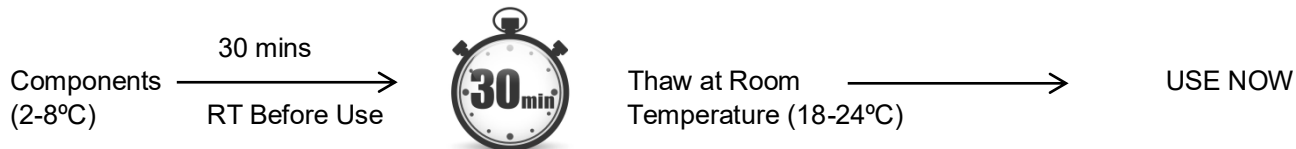
Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis...Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA...Arthritis Rheum...2003...Wiley

Immunogenicity of anti-TNF biologic therapies for rheumatoid arthritis...Atzeni F, Talotta R, Salaffi F, Cassinotti A, Varisco V, Battellino M...Autoimmun Rev...2013...Elsevier

Recommendations for the design and optimization of immunogenicity assays...Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher R, Finco-Kent D...J Pharm Biomed Anal...2008...Elsevier

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 Positive / Negative Controls / diluted Samples to the respective wells.

4. Cover plate and incubate for at 37°C.

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

6. Pipette **100 ul Adalimumab:HRP Conjugate** to all wells.

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.

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