KRIBIOLISA™ OVALBUMIN ELISA

REF : KBBP06

Ver3.0

RUO

Enzyme Immunoassay for the Quantification of Ovalbumin in biological preparations

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:

Egg allergies occur in a small size of the population. As some of the vaccines like influenza and yellow fever vaccines are made in eggs, egg proteins (primarily Ovalbumin) are present in the final product. Residual quantities of egg proteins found in the vaccines may induce severe and rarely fatal hypersensitivity reactions in children with egg allergies.

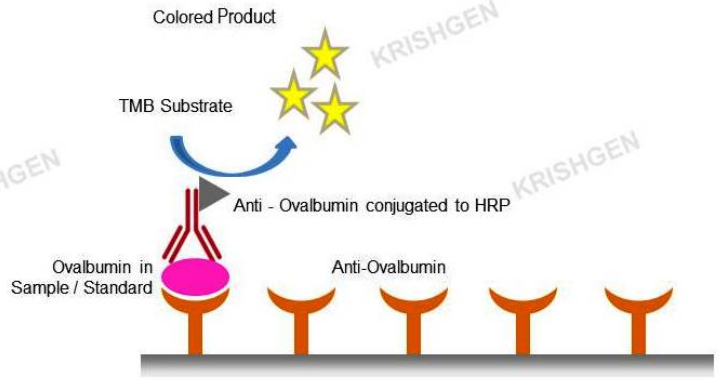
The KRIBIOLISA™ Ovalbumin ELISA enables estimation of Ovalbumin found as impurities in vaccines and cell culture supernatants. For egg-based vaccines, ovalbumin is the main impurity, corresponding to approximately 60% of the total protein content. Ovalbumin needs to be removed, as it can cause severe allergic reactions. According to WHO, the recommended ovalbumin content should be below 1 ug ovalbumin/vaccine dose.

Intended Use:

The KRIBIOLISA™ OVALBUMIN ELISA is a generic kit to be used for determining the presence of Ovalbumin in biological preparations.

Principle:

The method employs sandwich enzyme immunoassay technique. Anti-Ovalbumin antibodies are pre-coated onto microwells. Samples, Standards and Control are pipetted into microwells and Ovalbumin present in the sampleare bound by the capture antibodies. Then, HRP labeled Anti-Ovalbumin antibody is added to form an immune complex. After washing the microwells to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Ovalbumin present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

1. Anti-Ovalbumin Antibody Coated Microtiter Plate (8x12 wells) - 1 no
2. Ovalbumin Standards (0.625, 1.25, 2.5, 5.0, 10.0, 20.0 ng/ml) - 1 ml each
3. Positive Control – 1 ml
4. Anti-Ovalbumin:HRP Conjugated Antibody - 15 ml
5. (1X) Sample Diluent - 50 ml
6. (10X) Wash Buffer - 50 ml
7. TMB Substrate - 15 ml
8. Stop Solution - 15 ml
9. Instruction Manual

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 5000 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 as indicated on the component label.
2. Before using, bring all components to room temperature (18-25 ºC). Upon assay completion return all componentsto appropriate storage conditions.

Health Hazard Warnings:

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use only. Not for Diagnostic Use.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the Ovalbumin concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. High Dose Hook Effect may be observed in samples with very high concentrations of Ovalbumin, usually in samples from the initials stages of purifications. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of samples containing sodium azide (NaN_3), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of Ovalbumin.
4. All Standards, Positive Control and Samples should be assayed at least 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, Positive Control and Samples.

Specimen Collection and Handling:

This Ovalbumin kit is intended for the determination of the Ovalbumin content in diluted sample.

Test Sample Preparation - **Samples** have to be diluted 1:11 to 1:101 (v/v), e.g. for 1:11 (100 ul sample + 1000 ul **(1X) Sample Diluent**) prior to assay. Samples collected shouldn´t be stored longer than 48 hours at 2-8 °C. For long term storage,samples should be stored at -20°C. Avoid repeated freezing and thawing of the same sample. Frozen samples shouldbe warmed to room temperature and mixed thoroughly before testing.

Reagent Preparation:

1. Bring all reagents to room temperature prior to use.
2. To make (**1X) Wash Solution**, add **10 ml** of (**10X) Wash Buffer** in **90 ml** of DI water.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards, Control and Samples be run atleast in duplicates. A standard curve is required for each assay.
2. Add **100 ul** of **Anti-Ovalbumin:HRP Conjugate** to each well.
3. Pipette **100 ul** of **Standards, diluted Samples and Positive Control** into the respective wells.
4. Seal the plate and incubate for 60 minutes at Room Temperature.
5. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside downon absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interferein the reading step. All the washes should be performed similarly.
6. Pipette out **100 ul** of **TMB Substrate** in each well.
7. Seal the plate and incubate at Room Temperature for 15 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
8. Stop reaction by adding **100 ul** of **Stop Solution** to each well. Wells should turn from blue to yellow in color.
9. Read the absorbance at 450nm with a microplate reader within 30 minutes of stopping the reaction.

Calculation of Results:

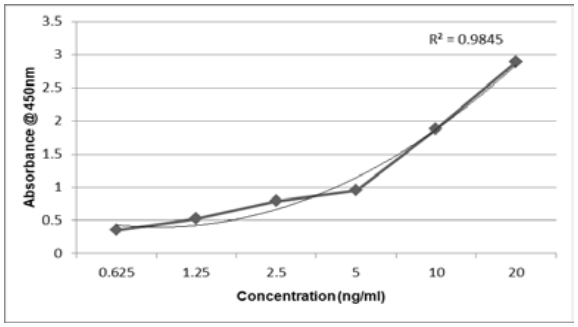
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. To determine the unknown Ovalbumin concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the Ovalbumin concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software like 4PL (2nd order) or cubic spline may be preferred.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs
0.625	0.352	0.359	0.356
1.25	0.528	0.523	0.526
2.5	0.788	0.789	0.789
5	0.945	0.951	0.948
10	1.873	1.878	1.876
20	2.873	2.901	2.887

Typical Graph



Abs = absorbance at 450nm

Interpretation of Results:

A test run is valid if:

1. the mean absorbance of standard 1 (20.0 ng/ml) is ≥ 1.50
2. the mean absorbance of standard 6 (0.625 ng/ml) is ≤ 0.50
3. the control is determined between 5.0 ng/ml and 10.0 ng/ml

Performance Characteristics:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:
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 found to be 0.34 ng/ml

Cross Reactivity
Albumin from the following species have been tested with Ovalbumin and showed no cross reactivity: Human Serum Albumin (HSA), Bovine Serum Albumin (BSA), Mouse Serum Albumin, Sheep Serum Albumin, Rabbit Serum Albumin

Precision:
Intra-Assay coefficient of variation (CV) in Ovalbumin from 10-fold determinations**.**

Sample No# (10 fold dilutions)	Mean Abs	Standard Deviation	Cumulative Variance (%)
1	2.354	0.058	2.31
2	1.520	0.035	2.28
3	0.788	0.022	3.12
4	0.498	0.016	3.09
5	0.299	0.009	3.19

Sample No# (10 fold dilutions)	Mean Concentration (ng/ml)	Standard Deviation	Cumulative Variance (%)
1	20.01	0.64	3.31
2	9.97	0.31	3.12
3	4.9	0.14	3.32
4	2.46	0.11	4.01
5	1.31	0.08	3.95

Inter-Assay coefficient of variation (CV) in Ovalbumin in 24 different test runs.

Sample No#	Mean Abs	Standard Deviation	Cumulative Variance (%)
1	2.421	0.154	6.14
2	1.456	0.213	5.86
3	0.891	0.231	7.65
4	0.432	0.142	8.1

Sample No#	Mean Concentration (ng/ml)	Standard Deviation	Cumulative Variance (%)
1	13.01	1.21	11.24
2	6.54	0.87	10.11
3	3.12	0.23	9.12
4	1.45	0.21	9.01

Dilutional Linearity:
Dilution linearity was performed to demonstrate that a sample with a spiked concentration above the ULOQ can be diluted to a concentration within the working range and offered acceptable recovery as per table below.

Sample No#	Dilution factor (DF)	Observed (ng/ml) x DF	Expected Concentration (ng/ml)	Recovery %
1	Neat	131.5	131.5	100
	1:2	149.9		114
	1:4	162.2		123
	1:8	165.4		126
2	Neat	128.7	128.7	100
	1:2	142.6		111
	1:4	139.2		108
	1:8	171.5		133

Safety Precautions:

- **This kit is for research use and in vitro testing 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 at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from 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 absenceof 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:

Huntington, J. A. and Stein, P.E. Structure and properties of ovalbumin. Journal of Chromatography B 756, 189–198 (2001)

WHO Technical Report Series No. 927 (2005)

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Inflexal® V—The influenza vaccine with the lowest ovalbumin content
O Kürsteiner, C Moser, H Lazar, P Durrer - Vaccine, 2006 - Elsevier

Ovalbumin content in 2009 to 2010 seasonal and H1N1 monovalent influenza vaccines
KH Waibel, R Gomez - Journal of allergy and clinical immunology, 2010 - jacionline.org

The development and standardization of an ELISA for ovalbumin determination in influenza vaccines
G Edevåg, M Eriksson, M Granström - Journal of biological standardization, 1986 - Elsevier

SCHEMATIC ASSAY PROCEDURE

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

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

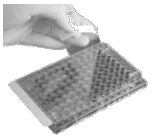
Thaw at Room Temperature (18-24ºC) → USE NOW

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 Anti-Ovalbumin:HRP Conjugate** into each well.

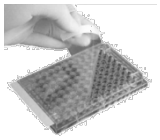
3.  Pipette **100 ul Standards** / diluted **Samples** / **Positive Control** into respective well.

4. Cover plate  and incubate for 

ıt Room Temperature.

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

6.  Pipette **100 ul TMB Substrate** into each well.

7. Cover plate  and incubate for 

at Room Temperature in dark. Protect from light.

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

9. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Ovalbumin equivalent
1A 2A	zero std zero std			
1B 2B	0.625 ng/ml 0.625 ng/ml			
1C 2C	1.25 ng/ml 1.25 ng/ml			
1D 2D	2.5 ng/ml 2.5 ng/ml			
1E 2E	5 ng/ml 5 ng/ml			
1F 2F	10 ng/ml 10 ng/ml			
1G 2G	20 ng/ml 20 ng/ml			
1H 2H	Sample Sample			
3A 4A	Sample Sample			

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SYMBOLS KEY

MTP	**Anti-Ovalbumin Coated Microtiter Plate (8x12 wells)**
STD	**Ovalbumin Standard**
HRP CONJ	**Conjugate Horseradish Peroxidase**
1X SAMP DIL	**(1X) Sample Diluent**
10X WASH BUF	**(10X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**