






# KRIBIOLISA™ OVALBUMIN ELISA

**REF** : KBBP06

Ver2.0

**RUO**

Immunoassay for the quantification of Ovalbumin

<b>RUO</b>	<b>For Research Use Only</b>	<b>REF</b>	<b>Catalog Number</b>
	<b>Store At</b>	<b>LOT</b>	<b>Batch Code</b>
	<b>Manufactured By</b>		<b>Biological Risk</b>
	<b>Expiry Date</b>		<b>Consult Operating Instructions</b>

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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.

**Intended Use:**

This generic kit is intended in determining the presence of Ovalbumin in biological fluids. A highly sensitive and specific Enzyme linked immunosorbent can be used for example to control the purity of vaccines.

**Principle:**

This assay is based on the Sandwich ELISA method. Samples containing Ovalbumin are reacted with microtiter wells pre-coated with affinity purified capture Ovalbumin antibody followed by washing and then detected using detection antibody and HRP Conjugated antibody. This immunological reaction results in formation of an immuno complex of solid phase. The wells are washed to remove any unbound reactants. The substrate 3, 3', 5, 5' Tetramethyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of Ovalbumin present.

**Materials Provided:**

1. Anti-Ovalbumin Coated Microtiter Plate (96 wells) – 1 no
2. Ovalbumin Standards – 2 vials
3. Detection antibody- 2 vials
4. HRP conjugated antibody- 2 vials
5. Wash Buffer (20X) – 30ml
6. Assay Diluent – 50ml
7. HRP conjugate diluent- 12ml
8. TMB Substrate – 12ml
9. Stop Solution – 12ml
10. 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 50ul to 5000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi-log graph paper or software for data analysis.
6. Timer.
7. Absorbent paper.

**Handling/Storage:**

1. Store main kit components at 2-8°C.
2. Store Ovalbumin standards and detection antibody at -20°C. Upon thawing, aliquot standards and antibody into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25 °C). Upon assay completion return all components to 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 or Manufacturing use only.

**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 initial 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<sub>3</sub>), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of Ovalbumin.
4. All Standards 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 and Samples.

**Reagent Preparation:**

**Please refer to lot specific instructions for preparation of the reagents.**

**Assay Procedure:**

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. Standards Dilution: Prepare the standards as per the table given below:

100 ng/ml	Standard No.7	10 µl Original Standard (10 µg /ml) + 990 µl Assay diluent
50 ng/ml	Standard No.6	500 µl Standard No.7 + 500 µl Assay diluent
25 ng/ml	Standard No.5	500 µl Standard No.6 + 500 µl Assay diluent
12.5 ng/ml	Standard No.4	500 µl Standard No.5 + 500 µl Assay diluent
6.25 ng/ml	Standard No.3	500 µl Standard No.4 + 500 µl Assay diluent
3.13 ng/ml	Standard No.2	500 µl Standard No.3 + 500 µl Assay diluent
0.5 ng/ml	Standard No.1	80 µl Standard No.2 + 420 µl Assay diluent

3. Pipette out 100µl of **Standards** and **Samples** into the respective wells as mentioned in the work list, **Assay Diluent** serves as the zero standard. Seal plate and incubate for 90 minutes at 37°C.
4. Aspirate and wash plate 5 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. All the washes should be performed similarly.
5. Pipette out 100µl of diluted **Detection Antibody** into each well. Cover the plate and incubate for 1 hour at 37°C.
6. Wash plate 5 times with **Wash Buffer (1X)** as in step 3.
7. Add 100µl of diluted **HRP-conjugate** solution to each well, seal plate and incubate for 30 minutes at 37°C.
8. Wash plate 5 times with **Wash Buffer (1X)** as in step 3. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
9. Pipette out 100µl of **TMB Substrate** in each well. Incubate the plate at 37°C for 15-30 minutes. Positive wells should turn bluish in color.
10. Stop reaction by adding 100µl of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
11. Read the absorbance at 450nm.

Example of a Work list

Well #	Contents	Abs at 450nm	Mean Absorbance
1A	Zero Std		
2A	Zero Std		
1B	0.5ng/ml		
2B	0.5ng/ml		
1C	3.13ng/ml		
2C	3.13ng/ml		
1D	6.25ng/ml		
2D	6.25ng/ml		
1E	12.5ng/ml		
2E	12.5ng/ml		
1F	25ng/ml		
2F	25ng/ml		
1G	50ng/ml		
2G	50ng/ml		
1H	100ng/ml		
2H	100ng/ml		

**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. Plot the standard curve on Semi-Log graph paper, with Ovalbumin concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. 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 may be preferred.

**Safety Precautions:**

- **This kit is for in vitro 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 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 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.

**LIMITED WARRANTY**

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