Instructions for Use

Serazym® Ovalbumin

Enzyme immunoassay for the quantitative determination of ovalbumin in biological fluids

REF E-041c-1 Σ 96

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Manufacturer	Date of manufacture	Use by date
LOT Batch number	REF Article number	SN Serial number
Keep away from sunlight	Temperature limits	Biological risks
Do not reuse	Consult instructions for use	Caution
	Sufficient for <n> tests	

Intended Use

Serazym® Ovalbumin is an enzyme immunoassay for the quantitative determination of ovalbumin in biological fluids like cell culture supernatants or vaccines by a laboratory professional user.
The test must not be used for human or veterinary diagnostics and by lay persons.

Principle of the Test

Serazym® Ovalbumin is an enzyme immunoassay based on polyclonal antibodies against ovalbumin. Samples, ready to use standards and control are dispensed simultaneously with peroxidase (HRP)-labeled polyclonal anti-ovalbumin antibodies into the wells of the microtiter plate coated with polyclonal anti-ovalbumin antibodies. After a 60-minute incubation at room temperature (RT, 20...25 °C) unbound components are removed by a wash step. HRP converts the colorless substrate solution to a blue reaction product in the following 15-minute enzymatic reaction step at RT. The reaction is stopped by the addition of stop solution, resulting in a color change from blue to yellow.
The optical density (OD) of the reaction product measured at 450 nm measuring filter and ≥ 620 nm reference filter is directly proportional to the concentration of specifically bound ovalbumin. Using the measured absorbances of the standards and their corresponding ovalbumin concentrations, a reference curve is generated from which the concentrations of unknown samples can be read.

Test Components (Delivery Scope)

			For 96 wells
1	**WELLS**	**Microtiter Plate** coated with polyclonal anti-ovalbumin antibodies (rabbit) Contains material of animal origin.	12 single breakable 8-well strips, colorless, vacuum-sealed with desiccant
2	**WASHBUF (10x)**	**Wash Buffer (10x)** Seramun® Wash buffer A TRIS-based buffer	50 mL concentrate for 500 mL solution, colorless, white cap
3	**DILUENT**	**Sample Diluent** Seramun® Sample diluent C TRIS-based buffer Contains material of animal origin.	50 mL, ready to use, colored red, black cap
4	**STD** **1 - 6**	**Standards 1 - 6** **STD 1 = 20 ng/mL** **STD 2 = 10 ng/mL** **STD 3 = 5.0 ng/mL** **STD 4 = 2.5 ng/mL** **STD 5 = 1.25 ng/mL** **STD 6 = 0.625 ng/mL** diluted ovalbumin Contains material of animal origin.	1.0 mL each, ready to use, colored red, colorless cap
5	**CONTROL**	**Control** diluted ovalbumin; concentration see Certificate of Analysis Contains material of animal origin.	1.0 mL, ready to use, colored red, green cap
6	**CONJ HRP**	**HRP Conjugate** HRP-labeled polyclonal anti-ovalbumin antibodies (rabbit) Contains material of animal origin.	15 mL, ready to use, colored green, red cap
7	**SUBSTR**	**Substrate** < 0.1 % 3,3',5,5'-tetramethylbenzidine; < 0.05 % hydrogen peroxide	15 mL, ready to use, colorless, blue cap

8	**STOP**	**Stop Solution** SeramunBlau® stop 0.25 M sulphuric acid	15 mL, ready to use, colorless, yellow cap
9	**COVER**	**Covering Film**	1 piece
10		**Certificate of Analysis**	1 piece
11		**Instructions for Use**	1 piece

Additional Materials and Aids Required for the Test Procedure

Adjustable single-channel micropipette • 8-channel micropipette or multi-channel micropipette with pipette tips • reagent container for multi-channel micropipettes • 8-channel wash comb with vacuum pump and waste bottle or microplate washer • microplate reader with 450 nm measuring filter and ≥ 620 nm reference filter • deionized water • measuring cylinder • tubes for sample preparation

Important Information

This product is for *in vitro* use only and may be used by laboratory professionals only.
Follow the instructions carefully.
The shelf life specified must be observed. Do not use reagents from damaged packages or bottles. Do not mix components with reagents from other manufacturers.
Mixing of test kit components of different lots is only allowed for wash buffer (10x), sample diluent, substrate and stop solution.

Information on Assay Procedure
All reagents should be stored at 2…8 °C. The stop solution may be stored at room temperature. Bring all test components to room temperature before use. Reagents that appear contaminated should not be used.
Each well of a microtiter plate can only be used once. For larger sample series, pipetting reagents from liquid reservoirs using a multi-channel micropipette is recommended to avoid time delays and contaminations. Each sample, standard and control should be pipetted with a new pipette tip.
Follow the pipetting scheme and time schedules of the protocol.

The aspiration and washing steps may be performed manually or with the help of a microplate washer or waterjet pump. In case of using a microplate washer, ensure that the wells are completely filled (at least 300 µL/well) and completely drained in each wash cycle. If washing is done manually, foam formation and air bubbles in the wells must be avoided. Wash solution should be allowed a minimum reaction time of 5 s in the wells per wash cycle. Remove wash buffer residues by thoroughly aspirating or tapping out the wells!
Protect substrate from light!

Safety Instructions
Do not swallow reagents and avoid contact with skin and mucous membranes.
Some reagents may contain biocides as preservative.

Control, standards, samples and waste should be considered potentially infectious and handled with caution. Used wash buffer, other test kit components, and all materials that have come into contact with samples and reagents should be collected in appropriate containers and disposed of according to local and national regulations.
Additional safety-relevant information may be taken from the Safety Data Sheet.

Limitations of the Procedure
False results may be caused by contamination of reagents or equipment with ovalbumin. Cross-reactions of reagents or specimens, bacterial or fungal contamination of reagents or specimens, incorrect washing and incorrect incubation times can also cause false results.

Due to the high-dose hook effect occurring in one-step assays, samples with ovalbumin concentrations ≥ 1.0 µg/mL may be determined too low. For this reason, it is recommended to use samples in two dilution levels that differ by at least a factor of 10.

Sample Treatment

Sample Collection
Collect samples in suitable sample container.

Preparation of Diluted Samples
Before testing appropriate dilutions of samples have to be prepared to generate measured values within the range of the reference curve.
Exemplary dilutions of samples range from 1 : 11 to 1 : 101.
Pipette 1,000 µL of sample diluent into a reaction tube. For a 1 : 11 dilution add 100 µL sample to the sample diluent, for a 1 : 101 dilution add 10 µL sample to the sample diluent and mix thoroughly.
Other dilution protocols have to be established individually by the user.

Shelf Life and Storage of Diluted Samples
Samples that have already been diluted in sample diluent may be stored at 2...8 °C and examined within 72 h. Alternatively, diluted samples may be stored at -20 °C. Repeated (> 3x) freezing and thawing of diluted samples should be avoided. Allow stored diluted samples to reach room temperature and mix thoroughly before testing.
Alternative storage conditions of diluted and undiluted samples may be determined individually by the user.

Reagent Treatment

Reagent Shelf Life and Storage
The complete test kit with sealed reagent bottles and 8-well strips can be stored at 2...8 °C until the printed expiry date. All opened test kit components are stable for up to 2 months when stored properly at 2...8 °C. The diluted wash buffer can be stored at 2...8 °C for up to 1 month.

Reagent Preparation
All reagents must have reached room temperature before being used in the assay. The microtiter plate with breakable 8-well strips is vacuum sealed with desiccant. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored at 2…8 °C and protected from moisture in the original cover carefully resealed.
The sample diluent is ready to use. If stored at 2...8 °C, precipitates may occur which dissolve again after reaching room temperature.

Dilute wash buffer (10x) 1 : 10 with deionized water before use.
Example: 10 mL wash buffer (10x) + 90 mL deionized water.

Assay Procedure

One test kit (1x 96 wells) allows the quantitative determination of ovalbumin in up to 40 samples when samples, standards and control are run in duplicate.

1. Allow test reagents and required number of wells to reach room temperature (RT, 20…25 °C). Shake reagents gently before use. Avoid foaming.
2. Pipette 100 µL **CONJ HRP** HRP conjugate per well.
3. Add 100 µL **diluted sample**
   100 µL **STD** **1 - 6** standards 1 - 6
   100 µL **CONTROL** control each and mix gently.
4. Cover the plate and incubate for 60 min at RT.
5. Decant, then wash each well 5x with 300 µL diluted wash buffer.
   Tap dry onto absorbent paper if necessary.
6. Add 100 µL **SUBSTR** substrate per well.
7. Incubate for 15 min at RT **protected from light**.
8. Add 100 µL **STOP** stop solution per well, mix gently.
9. Read OD at 450 nm measuring filter and ≥ 620 nm reference filter with a microplate reader within 30 min following reaction stop.

Evaluation of Results

A reference curve is created by plotting the measured absorbances of the standards STD 1 - 6 (y-axis) against the corresponding ovalbumin concentrations (x-axis).
The 4-parameter regression model is recommended to create the reference curve.

The absorbance of an unknown sample is converted into an ovalbumin concentration [ng/mL] by extrapolating from the reference curve. Samples with higher ovalbumin concentrations than standard STD 1 (20 ng/mL), should be retested after further dilution. In case of pre-dilution of the samples, multiply the obtained concentration by the dilution factor.

Interpretation of Results

Test Validation
The test run is valid, if:

- mean absorbance $OD_{450/620nm}$ of standard STD 1 (20 ng/mL) ≥ 1.50,
- mean absorbance $OD_{450/620nm}$ of standard STD 6 (0.625 ng/mL) ≤ 0.50 and
- control is determined between 5.0 ng/mL and 10 ng/mL.

If one of the above-mentioned quality criteria is not met, test should be repeated strictly following the test procedure (correct sample and wash buffer dilution, correct preparation of reagents, correct incubation times and temperatures, careful washing, etc.). In case of repeated failure of the quality criteria contact the manufacturer.

Performance Characteristics

Precision
For the determination of the intra-assay coefficient of variation (CV) samples were measured in a 12-fold determination in one test run:

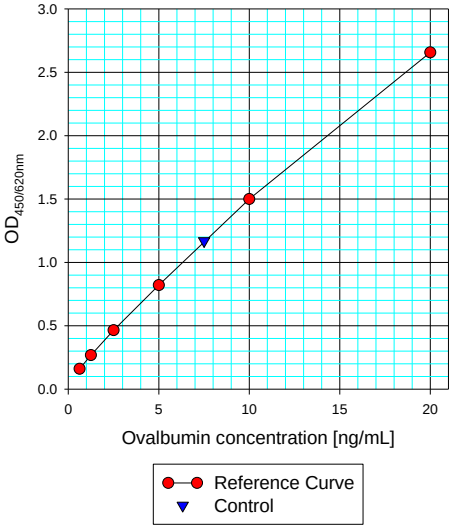
Sample	Intra-assay coefficient of variation			
	$\bar{x}$ OD	CV [%]	Concentration [ng/mL]	CV [%]
1	2.494	2.3	19.9	3.3
2	1.513	2.3	10.1	3.1
3	0.867	2.6	4.9	3.3
4	0.509	3.1	2.5	3.9
5	0.303	2.9	1.3	3.0
6	0.199	4.2	0.7	6.7

The inter-assay coefficient of variation in Serazym® Ovalbumin was determined in 24 individual test runs:

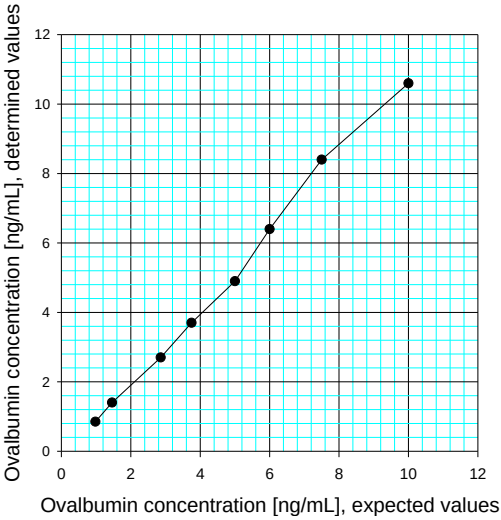
Sample	Inter-assay coefficient of variation			
	$\bar{x}$ OD	CV [%]	Concentration [ng/mL]	CV [%]
1	2.319	5.6	20.0	7.8
2	1.387	8.0	10.1	10.1
3	0.781	8.1	5.0	9.5
4	0.407	7.5	2.4	8.1

Cross-reactivity
Albumins from the following species showed no cross-reactivity when tested in Serazym® Ovalbumin: Human serum abumin (HSA), bovine serum albumin (BSA), mouse serum albumin, sheep serum albumin and rabbit serum albumin.

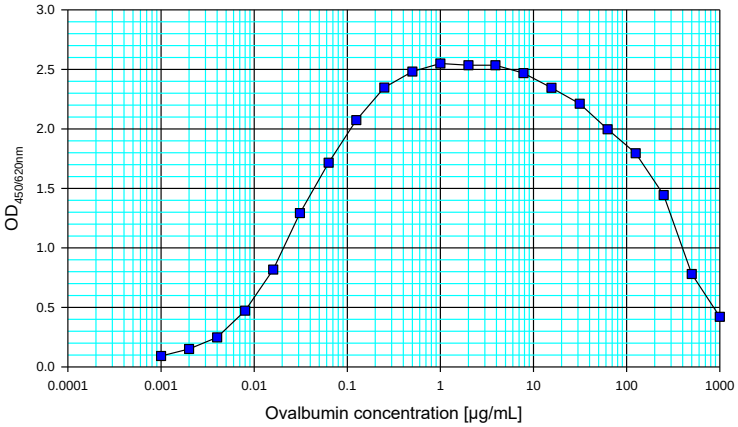
Reference Curve
A typical reference curve of Serazym® Ovalbumin is shown below:



Linearity of Dilution
Titration of the ovalbumin standard with concentrations between 0.98 ng/mL and 10 ng/mL, with measured and calculated ovalbumin concentrations plotted against each other:

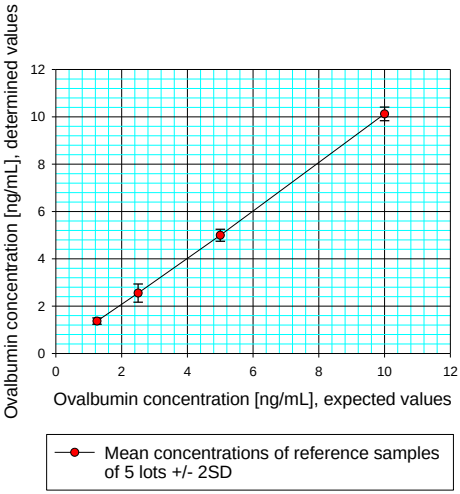


High-dose Hook Effect
To determine the high-dose hook effect occurring in one-step assays (simultaneous incubation of samples and conjugate), ovalbumin concentrations between 0.001 µg/mL and 1,000 µg/mL were determined.
In order to meet the measuring range of the photometer 100 µL of the final volume (= 200 µL) were removed from the wells after the reaction was stopped before the measurement. After volume reduction the dose dependent signal intensity reveals a high-dose hook effect beginning with an ovalbumin concentration of ≥ 1.0 µg/mL.



Recovery

For the determination of the recovery ovalbumin reference samples with theoretical concentrations of 10.0 ng/mL, 5.0 ng/mL, 2.5 ng/mL and 1.25 ng/mL were tested in five different lots. The lot specific standards and the ovalbumin reference samples were run in duplicate. The mean absorbance of each standard concentration was used to generate a reference curve, from which the concentrations of the different reference samples were determined. The calculated concentrations of the reference samples determined in the five different lots were plotted against their expected concentrations.
Error bars demonstrate the 2-fold standard deviation (± 2SD).



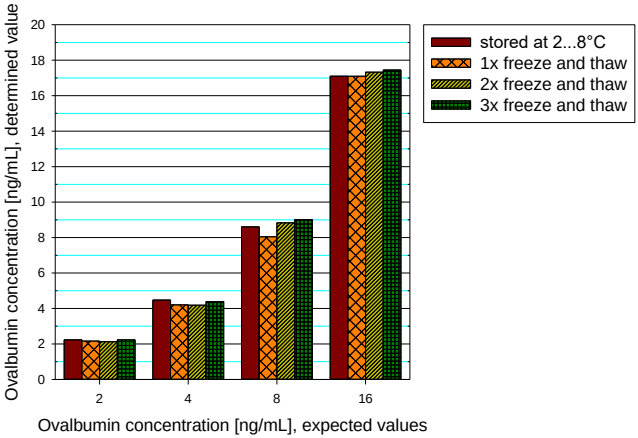
Expected ovalbumin concentration [ng/mL]	Determined ovalbumin concentration [ng/mL]	Recovery [%]
10.0	10.1	101.0
5.0	5.0	100.0
2.5	2.55	102.0
1.25	1.37	109.6

The recovery of the ovalbumin references in five different lots has been determined within the range of 80 - 120 % according to ICH guidelines.

Impact of Freezing and Thawing

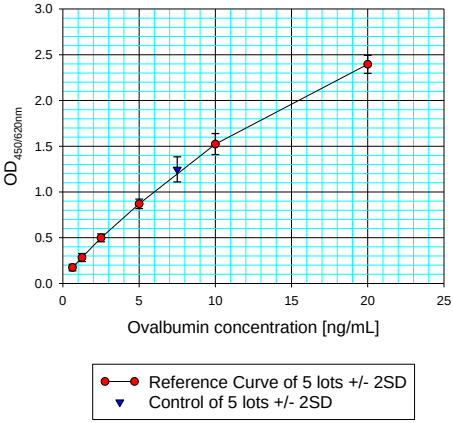
Ovalbumin reference samples with concentrations of 16 ng/mL, 8.0 ng/mL, 4.0 ng/mL and 2.0 ng/mL were split into 4 aliquots. One aliquot was stored at 2…8 °C. The remaining three aliquots were frozen and thawed once, twice and three times. After three days, all aliquots were tested in duplicate in one test run.

All samples were determined in the range of ± 2SD regardless of the number of freeze-thaw-cycles.



Lot-to-lot Consistency

Averaged reference curve of five different lots with error bars demonstrating the 2-fold standard deviation (± 2SD) is shown below:



Change History

Version	Section	Modifications
2024-08 (v01)	Entire document	Update of Intended Use Update of Performance Characteristics Revision of section structure Layout and editorial changes