Serazym® Ovalbumin

Enzyme-linked immunosorbent assay for detection of ovalbumin in biological fluids

REF E-041a-1 Σ 96 REF E-041a-2 Σ 2x 96 IVD *In-vitro*- diagnostic test CE

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Intended Use

The *Serazym*® Ovalbumin is an *in-vitro*- diagnostic test developed for very sensitive determination of ovalbumin in biological fluids and can be used for example to control the purity of certain vaccines (e.g. influenza).

Principle Of The Test

The *Serazym*® Ovalbumin is a fast immune enzymometric one-step assay based on affinity purified polyclonal antibodies (rabbit) against ovalbumin. Specimens and horseradish peroxidase (HRP) labelled anti-ovalbumin-antibodies are dispensed into the microtitration plate wells, which are coated with anti-ovalbumin-antibodies and are incubated simultaneously at 37°C / 99°F for 60 minutes. After the incubation unbound components are removed by washing the wells five times with wash buffer. Then substrate solution (tetramethylbenzidine and hydrogen peroxide) is added to each well and incubated for 10 minutes at 20…25°C / 68…77°F protected from light. The presence of specifically bound enzyme labelled antibodies in the wells is indicated by the development of a blue colour. Reaction stop by addition of sulphuric acid to the wells results in a colour change to yellow. Absorbances are read at 450 nm wavelength. For optimum results a reference filter (620 - 690 nm wavelength) should be used. A standard curve is created by plotting the produced absorbances of the different ovalbumin standards versus their concentrations. The absorbances of the unknown samples can be transformed into their corresponding ovalbumin concentrations by reading from the standard curve.

Test Components

			For 96 Wells	For 2x 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-ovalbumin-antibodies (rabbit)	12 single breakable 8-well strips vacuum-sealed with desiccant	2x 12 single breakable 8-well strips vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	50 ml concentrate for 500 ml solution white cap	2x 50 ml concentrate for 2x 500 ml solution white cap
3	DIL	**Sample diluent**	50 ml · ready to use coloured red black cap	2x 50 ml · ready to use coloured red black cap
4	STD 1-7	**Standard 1 – 7** S1 = 20.0 ng/ml; S2 = 10.0 ng/ml; S3 = 5.0 ng/ml; S4 = 2.5 ng/ml; S5 = 1.25 ng/ml; S6 = 0.625 ng/ml; S7 = 0.31ng/ml	1.0 ml per standard ready to use coloured red transparent cap	2x 1.0 ml per standard ready to use coloured red transparent cap
5	CONTROL	**Control** 7.5 ng/ml	1.0 ml · ready to use coloured red green cap	2x 1.0 ml · ready to use coloured red green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, polyclonal anti-ovalbumin-antibodies (rabbit) in protein stabilizer solution	15 ml · ready to use coloured green red cap	2x 15 ml · ready to use coloured green red cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap	2x 15 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap	2x 15 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage

The *Serazym*® Ovalbumin is intended for the determination of the ovalbumin content in diluted biological fluids. Samples should not be stored longer than 48 hours at 2...8°C / 36…46°F prior to use. Otherwise a storage temperature of -20°C / -4°F is recommended. Frozen samples should be rapidly warmed to room temperature and mixed thoroughly before testing. Repeated freezing and thawing of samples should be avoided.

Preparation

Dilute samples with sample diluent *(3)* usually 1 : 11 to 1 : 101. Pipette 1000 µl of sample diluent into a clean tube and add 100 µl (1 : 11) or 10 µl (1 : 101) of the sample. After it mix the solution gently.

Materials Required But Not Provided

Adjustable one-channel micropipette 0.100 - 1.000 ml and 0.010 - 0.100 ml · Adjustable 8-channel micropipette 0.050 - 0.200 ml · Pipette tips · Graduated measuring flasks 10 ml and 100 ml · Eppendorf tubes 2.0 ml · Microplate washer (automatic or hand wash head) · Microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference · Reagent containers for dispensing with 8-channel pipette · Distilled or deionized water · Stop-watch

Preparation And Storage Of Reagents

Kit size and expiry
One testkit E-041a-1 (1x 96 wells) and E-041a-2 (2x 96 wells) enables ovalbumin quantification in a maximum of 40 samples and 80 samples respectively when samples, standards and control are run in duplicate. The complete testkit with unopened reagent bottles and microtitration strips is stable until the expiry date printed on the kit box in case of storage at 2... 8°C / 36...46°F. Once opened all testkit components are stable for up to 2 months under appropriate storage conditions (2...8 °C / 36…46°F). When stored at 2...8°C / 36…46°F the ready to use wash solution can be used for at least 30 days.

Reagent preparation
Allow all components to reach room temperature prior to use in the assay. The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed. Prepare a sufficient amount of wash solution by diluting the 10fold concentrated wash buffer 1 + 9 with distilled or de-ionized water.
For example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Assay Procedure

Dilute samples with sample diluent *(3)* (usually 1 : 11 to 1 : 101).
Avoid any time shift during dispensing of reagents and samples.
Attention: due to the „high-dose hook effect" in one-step assays very high ovalbumin concentrations may be falsely determined too low. Therefore it is recommended to investigate samples in two dilutions differing at least about factor 10.
In case of using a washer make sure that the wells are completely filled (at least 300 µl / well) and drained in every single washing cycle. In case of manual washing avoid foam and air bubbles in the wells. It is recommended to tap the plate onto absorbent paper after each washing cycle.
Make sure that the soak time of the wash solution in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
Avoid light exposure of the TMB substrate solution!

Working steps

1. Warm all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense 100 µl **CONJ HRP** HRP-conjugate *(6)* per well and
3. Pipette: 100 µl **diluted samples**
   100 µl **STD 1 – 7** standard S1-S7 *(4)*
   100 µl **CONTROL** control *(5)*
   into the intended wells, mix gently.
   It is recommended to run samples, standards and control in duplicate.
4. Cover plate and incubate for 60 min at 37°C / 99°F.
5. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper.
6. Dispense 100 µl **SUBSTR TMB** substrate *(7)* per well.
7. Incubate for 10 min at room temperature (20…25°C / 68…77 °F) protected from light.
8. Dispense 100 µl **STOP** stop solution *(8)* per well, mix gently.
9. Read OD at 450 nm/ ≥ 620 nm with a microplate reader within 30 min after reaction stop.

Result Interpretation

Calculate the mean absorbance of standards, control and samples. Create a reference curve from the mean absorbances of the standards S1 – S7 (y-axis) and the corresponding ovalbumin concentrations of these standards (x-axis). Determine the ovalbumin concentration of the unknown samples by referring their mean absorbances to the reference curve and multiply the values with the dilution factor. Samples with ovalbumin concentrations exceeding those of standard S 1 (20 ng/ml) should be retested in higher predilution (1 : 101 or higher if necessary).

Reference Values

Test validity

A test run is valid if:

- the mean absorbance of standard 1 (20.0 ng/ml) is ≥ 1.50
- the mean absorbance of standard 7 (0.31 ng/ml) is ≤ 0.50
- the control is determined between ≥ 5.0 ng/ml and ≤ 10.0 ng/ml

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is correct (incubation times and temperatures, sample and wash buffer dilution, washing steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of the procedure

False results may be caused by cross contaminations of the kit reagents and samples, bacterial or fungal contaminations of reagents and/or samples, incorrect washing, incorrect incubation times and incorrect handling of samples and standards.

Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Ovalbumin from 12-fold determinations.

sample	mean OD	standard deviation	CV (%)
1	2.508	0.081	3.23
2	1.937	0.039	2.00
3	1.573	0.066	4.20
4	1.135	0.042	3.74
5	0.645	0.025	3.83
6	0.281	0.013	4.48

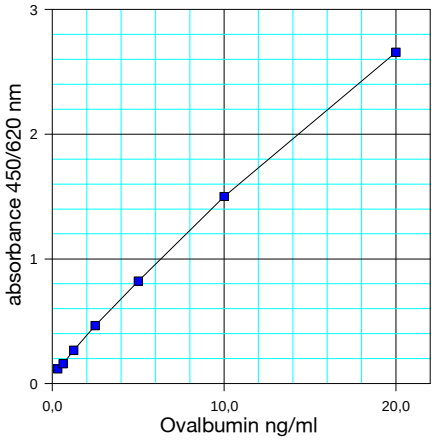
sample	mean concentration [ng/ml]	standard deviation	CV (%)
1	5.07	0.23	4.54
2	4.09	0.31	7.58
3	3.28	0.26	7.93
4	2.18	0.06	2.75
5	1.59	0.068	4.28
6	0.78	0.028	3.59

Inter-assay coefficient of variation (CV) in the *Serazym*® Ovalbumin in 24 different test runs.

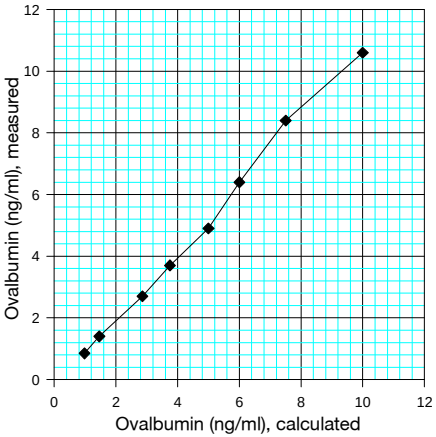
sample	mean OD	standard deviation	CV (%)
1	2.326	0.141	6.06
2	1.750	0.069	3.94
3	1.078	0.053	4.92
4	0.407	0.031	7.62

sample	mean concentration [ng/ml]	standard deviation	CV (%)
1	8.83	0.73	8.27
2	4.55	0.29	6.37
3	3.04	0.21	6.91
4	0.82	0.05	6.10

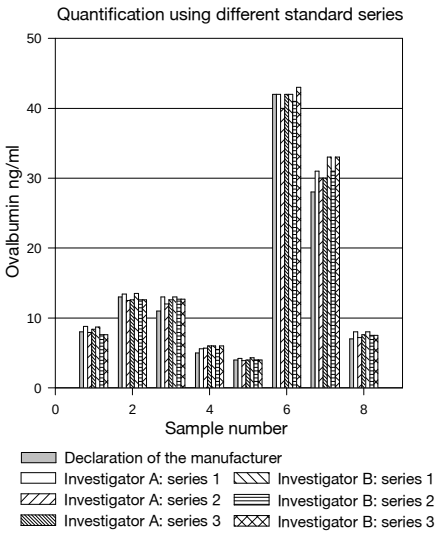
Typical calibration curve of the *Serazym*® Ovalbumin



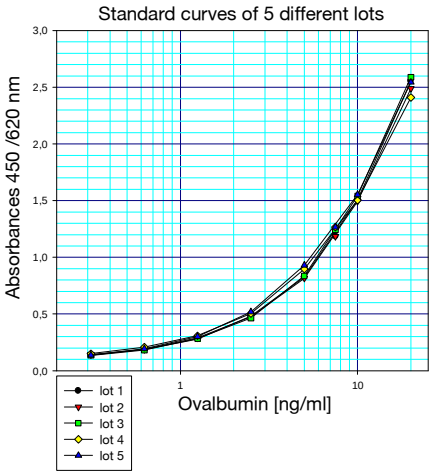
Dilution linearity



Ovalbumin concentration in vaccines

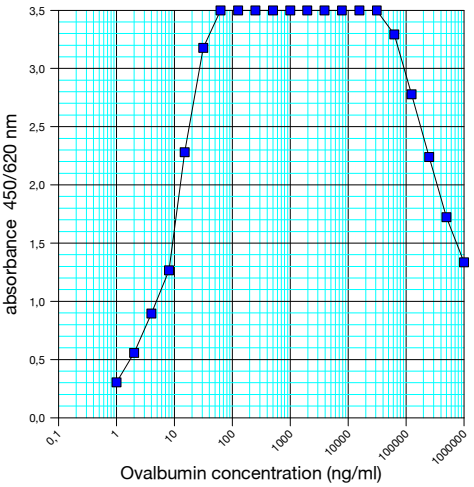


Lot-to-lot consistency



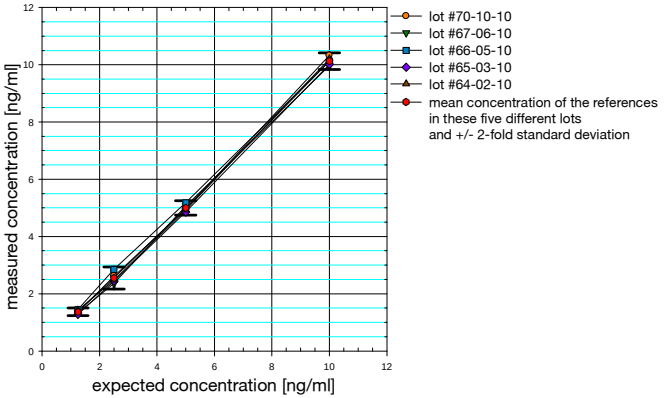
High-Dosis-Hook-Effect

Development of signal intensity in dependence of the Ovalbumin concentration reveals a high-dosis-hook-effect which starts by exceeding a concentration of about 40 µg Ovalbumin/ml.

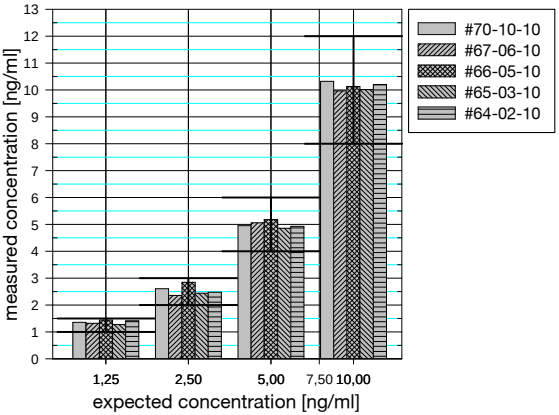


Recovery

For the determination of recovery ovalbumin reference samples with theoretical concentrations of 10.00 ng/ml, 5.00 ng/ml, 2.50 ng/ml and 1.25 ng/ml were run in 5 different lots of E-041a. The lot specific standards and the ovalbumin reference samples were run in duplicate. The mean absorbance of the twofold determination of each standard concentration was used to generate the standard curves from which the absorbances of the different references were converted into concentrations. The calculated concentrations of the reference samples determined in the 5 different lots were plotted against their theoretical concentrations. Measured vs. expected concentrations of the different references. Error bars demonstrate the twofold standard deviation (+/-2s).

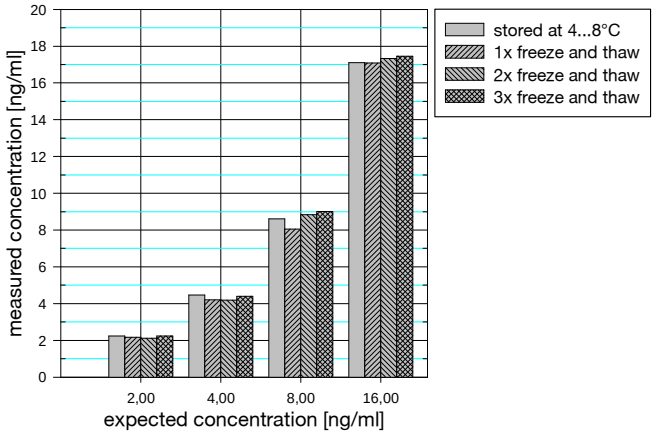


Recovery of the different references in 5 different lots indicating the interval of ± 20 percent [80 – 120% recovery] according to the ICH guidelines represented by error bars.



Influence of freeze and thaw

Ovalbumin reference samples with theoretical concentrations of 16.00 ng/ml, 8.00 ng/ml, 4.00 ng/ml and 2.00 ng/ml were aliquoted into 4 portions. One aliquot was stored at 4…8°C. The remaining 3 portions were subjected to one, two and three freeze-thaw-cycles resp. After 3 days all aliquots were thawed and tested as twofold determination within one run.



As a result the mean concentrations of all samples are within the range of ± 2-fold standard deviation irrespective of the number of freeze/thaw cycles.

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only. The expiry date on the respective labels is appropriate to note. The same applies for the specified shelf life of the reconstituted reagents. **Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.** Do not use reagents from other manufacturers. Precisely adhere to the prescribed incubation times and temperatures. Avoid time shift during dispensing of reagents. All reagents should be kept at 2…8°C / 36…46°F before use. Prepare a data sheet that defines the positions of samples, standards and control before starting the assay. Some of the reagents (2, 3, 4, 5, 6, 7) contain small amounts of Thimerosal (< 0.1% w/v) and Kathon (1.0% v/v) as preservative. They may neither be swallowed nor come into contact with skin or mucous membranes. In case of contact rinse with plenty of water. The stop solution contains 0.25 mol/l sulphuric acid. Avoid contact with skin and mucous membranes. In case of contact rinse with plenty of water. Handle all samples as potentially infectious. Use a new pipette tip for each sample to avoid contaminations. Since the kit contains potentially hazardous materials, the following precautions must always be observed:

Do not smoke, eat or drink while handling kit material!
Always use protective gloves!
Never pipette material by mouth!
Note safety precautions of the single test components!

Incubation Scheme *Serazym*® Ovalbumin (E-041a)

1.		100 µl	CONJ HRP *(6)*
		+	
		100 µl	**diluted sample**
		100 µl	STD S1 – S7 *(4)*
		100 µl	CONTROL *(5)*
		60 min	incubation at 37°C / 99°F
		5 x wash	with wash solution
2.		100 µl	SUBSTR TMB *(7)*
		10 min	incubation at room temperature, protected from light
3.		100 µl	STOP *(8)*

Read OD at 450 / ≥ 620 nm

 Manufacturer REF Catalogue-No. LOT Lot-No.  Number of determinations  Biohazard
 Notice advices  Use by  Storage temperature  Consult instructions for use