Instructions For Use

Serazym® Bovine Serum Albumin

Enzyme-linked immunosorbent assay for detection of bovine serum albumin in biological fluids

REF E-048 Σ 96 IVD *In-vitro*- diagnostic test CE

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Intended Use

The *Serazym*® Bovine Serum Albumin is an *in-vitro*-diagnostic test for the detection of bovine serum albumin (BSA) in biological fluids.

Principle Of The Test

The *Serazym*® Bovine Serum Albumin is a fast immune enzymometric two-step assay based on affinity purified polyclonal antibodies (rabbit) against bovine serum albumin (BSA). Specimens are dispensed into the microplate wells, that are coated with anti-bovine serum albumin antibodies and incubated at 20…25 °C / 68…77 °F for 60 minutes. After the incubation unbound components are removed by washing the wells five times with wash buffer. In the next step horseradish peroxidase (HRP) labelled anti-BSA-antibodies are dispensed and incubated at 20…25 °C / 68…77 °F for 60 minutes. Unbound conjugated antibodies are removed by washing.
Then substrate solution (tetramethylbenzidine and hydrogen peroxide) is added to each well and incubated for 15 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 optimal results a reference filter (620 - 690 nm wavelength) should be used. A standard curve is created by plotting the absorbances of the different BSA standards versus their concentrations. The absorbances of the unknown samples can be transformed into their corresponding BSA concentrations by reading from the standard curve.

Test Components

			For 96 Wells
1	WELLS	**Microtitration plate** coated with polyclonal anti-bovine serum albumin- antibodies (rabbit)	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
3	DIL	**Sample diluent**	70 ml · ready to use coloured red black cap
4	STD 1 - 6	**Standard 1-6** S1 = 500.0 ng/ml; S2 = 250.0 ng/ml; S3 = 125.0 ng/ml; S4 = 60.0 ng/ml; S5 = 30.0 ng/ml; S6 = 15.0 ng/ml	1.0 ml per standard ready to use coloured red transparent cap
5	CONTROL	**Control** 200 ng/ml	1.0 ml · ready to use coloured red green cap
6	CONJ HRP 101x	**HRP-conjugate** 101-fold HRP-labelled, polyclonal anti- bovine serum albumin - antibodies (rabbit)	0.3 ml · concentrate coloured red brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 ml · ready to use yellow cap

Preparation And Storage Of Samples

Collection and storage

The *Serazym*® Bovine Serum Albumin is intended for the determination of the bovine serum albumin content in diluted biological fluids. The dilution varies in dependence from the material. Samples should not be stored longer than 48 hours at 2...8 °C / 35.6 … 46.4 °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 : 6 to 1 : 21).

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 (1x96 wells) enables bovine serum albumin quantification in a maximum of 41 samples 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 / 35.6…46.4 °F. Once opened all testkit components are stable for up to 2 months under appropriate storage conditions 2...8 °C / 35.6…46.4 °F. When stored at 2...8 °C / 35.6…46.4 °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.

Sample diluent
The sample diluent is ready to use. During storage at 2…8 °C precipitates may occur, that will dissolve when the solution is warmed to room temperature. Make sure the sample diluent has reached room temperature before starting the assay.

Wash solution
Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer 1 + 9 with distilled or deionized water.
For Example: 10 ml wash buffer concentrate *(2)* + 90 ml distilled or deionized water.

Conjugate solution
Dilute the concentrated anti-BSA-HRP conjugate 101-fold *(6)* 1 + 100 with sample diluent *(3)*, 100 µl conjugate + 10.0 ml sample diluent. Prepare 1.0 ml conjugate solution for each test strip. For a full used plate 12.0 ml conjugate solution is required. Prepare the conjugate solution at least 15 min prior to use. The conjugate solution is stable for 1 day at room temperature.

Assay Procedure

Dilute samples with sample diluent *(3)* (usually 1:6 to 1:21). Avoid any time shift during dispensing of reagents and samples.

Attention: The *Serazym*® Bovine Serum Albumin is a very sensitive assay detecting only 15 ng BSA per ml sample material. It is recommended to use disposable reagent containers for pipetting the reagents. Make sure that the glassware used for buffer preparation is absolutely free of BSA. Any contamination of working equipment should be strictly avoided! Therefore special dispensers, pipettes and washers have to be used only for this test and not for other ELISAs.

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 **STD 1 – 6** standard S1-S6 *(4)*
   100 µl **CONTROL** control *(5)*
   100 µl **diluted samples**
   into the intended wells, mix gently.
   It is recommended to run samples, standards and control in duplicate.
3. Cover plate and incubate for 60 ± 1 min at 20…25°C / 68…77°F.
4. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper.
5. Dispense 100 µl **conjugate solution** (diluted from *(6)*) per well.
6. Cover plate and incubate for 60 ± 1 min at 20…25°C / 68…77°F.
7. Decant, then wash each well 5x with 300 µl wash solution (diluted from *(2)*) and tap dry onto absorbent paper.
8. Dispense 100 µl **SUBSTR TMB** substrate *(7)* per well.
9. Incubate for 15 min at 20…25°C / 68…77°F, protected from light.
10. Dispense 100 µl **STOP** stop solution *(8)* per well, mix gently.
11. Read absorbance 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 – S6* (y-axis) and the corresponding bovine serum albumin concentrations of these standards (x-axis). Plot the mean absorbances of the calibrator S1 – S6 (y-axis) against the corresponding BSA concentrations of these standards (x-axis). Determine the BSA concentration of the unknown samples by referring their mean absorbances to the reference curve. Samples with BSA concentrations exceeding those of standard S 1 (500 ng/ml) should be retested in higher predilution. In case of sample predilution values have to be multiplied with the dilution factor.

Reference Values

Test validity
A test run is valid if:

- the mean absorbance of standard 1 (500.0 ng/ml) is ≥ 1.50
- the mean absorbance of standard 6 (15.0 ng/ml) is ≤ 0.50
- the control is determined between 150 ng/ml and 250 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
Incorrect results may be caused by contamination of reagents or working equipment with BSA.
Cross contaminations of the kit reagents and samples, bacterial or fungal contaminations of reagents and/or samples, incorrect washing and incorrect incubation times may also cause erroneous results.

Performance Characteristics

Precision
Intra-assay coefficient of variation (CV) in the *Serazym*® Bovine Serum Albumin from 8-fold determinations of samples.

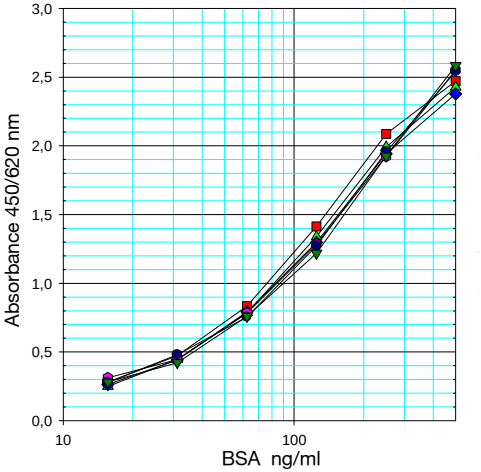
sample	mean absorbance	standard deviation	CV [%]
1	2.594	0.061	2.33
2	2.179	0.064	2.92
3	1.556	0.056	3.60
4	1.226	0.038	3.14
5	0.744	0.050	6.76
6	0.358	0.020	5.49

sample	mean concentration [ng/ml]	standard deviation	CV [%]
1	499	46.2	9.25
2	291	21.9	7.54
3	123	6.8	5.55
4	101	4.7	4.66
5	50.3	4.6	9.16
6	24.7	2.5	9.94

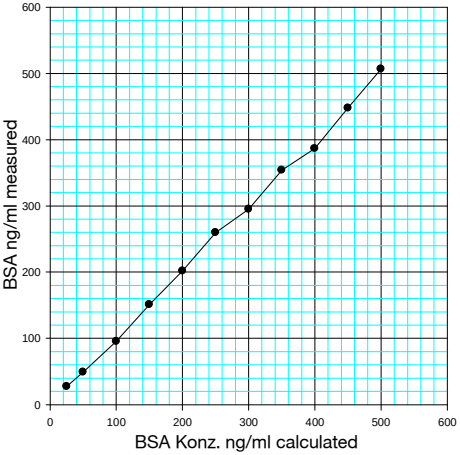
Inter-assay coefficient of variation (CV) in the *Serazym*® Bovine Serum Albumin in 12 different test runs.

sample	mean concentration [ng/ml]	standard deviation	CV [%]
1	419	42.0	10.03
2	313	23.6	7.56
3	195	16.2	8.31
4	97	5.7	5.89
5	51	3.8	7.44
6	29	2.6	9.06

Typical calibration curve in the *Serazym*® Bovine Serum Albumin



Dilution linearity



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*® Bovine Serum Albumin (E-048)

1.		100 µl	**STD S1 – S6** *(4)*
		100 µl	**CONTROL** *(5)*
		100 µl	**diluted sample**
		60 min	incubation (20…25°C / 68…77°F)
		5 x wash	with wash solution
2.		100 µl	**conjugate solution** (diluted from*(6)*)
		60 min	incubation (20…25°C / 68…77°F)
		5 x wash	with wash solution
3.		100 µl	**SUBSTR TMB** *(7)*
		15 min	incubation (20…25°C / 68…77°F) protected from light
4.		100 µl	**STOP** *(8)*

Read absorbances at 450 / ≥ 620 nm

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