

Serazym[®] Bovine Serum Albumin

Enzyme immunoassay for the quantitative determination of bovine serum albumin in biological fluids

REF E-048

 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® Bovine Serum Albumin is an enzyme immunoassay for the quantitative determination of bovine serum albumin (BSA) 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® Bovine Serum Albumin is an enzyme immunoassay based on polyclonal antibodies against bovine serum albumin (BSA). Samples, ready to use standards and control are dispensed into the wells of the microtiter plate coated with polyclonal anti-BSA antibodies. After a 60-minute incubation at room temperature (RT, 20...25 °C) unbound components are removed by a wash step. In the second incubation step horseradish peroxidase (HRP)-labeled anti-BSA antibodies were dispensed into the wells of the microtiter plate. After a 60-minute incubation at RT unbound conjugate is removed by a wash step. HRP converts the colorless substrate solution to a blue reaction product in the following 15-minute enzymatic reaction step. 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 BSA. Using the measured absorbances of the standards and their corresponding BSA concentrations, a reference curve is generated from which the concentration of a sample can be read.

Test Components (Delivery Scope)

		For 96 wells
1	WELLS	12 single breakable 8-well strips, colorless, vacuum-sealed with desiccant
2	WASHBUF (10x)	50 mL concentrate for 500 mL solution, colorless, white cap
3	DILUENT	70 mL, ready to use, colored red, black cap
4	STD 1 - 6	1.0 mL each, ready to use, colored red, colorless cap
	STD 1 = 500 ng/mL	
	STD 2 = 250 ng/mL	
	STD 3 = 125 ng/mL	
	STD 4 = 60 ng/mL	
	STD 5 = 30 ng/mL	
	STD 6 = 15 ng/mL	
	diluted BSA	
	Contains material of animal origin.	
5	CONTROL	1.0 mL, ready to use, colored red, green cap
	diluted BSA; concentration see Certificate of Analysis	
	Contains material of animal origin.	
6	CONJ (101x)	0.3 mL concentrate, colored red, brown cap
	HRP Conjugate	
	HRP-labeled polyclonal anti-BSA antibodies (rabbit)	
	Contains material of animal origin.	

7	SUBSTR	Substrate SeramunBlau® fast < 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	2 pieces
10	RESERVOIR	Disposable Reagent Container	3 pieces
11		Certificate of Analysis	1 piece
12		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 can also 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.

Attention: Serazym® Bovine Serum Albumin is a sensitive test, thus it is recommended to use disposable reagent containers when pipetting reagents. Any contamination of the working materials with BSA must be strictly avoided. Glassware used to prepare wash buffer and HRP conjugate, and equipment used for test processing must be BSA-free.

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 μ L/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 BSA. 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.

Sample Treatment

Specimen Materials

Biological fluids.

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 : 6 to 1 : 21.

Pipette 1,000 μ L of sample diluent into a reaction tube. For a 1 : 6 dilution add 200 μ L sample to the sample diluent, for a 1 : 21 dilution add 50 μ L 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 48 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. Do not use the sample diluent until it has reached room temperature.

Dilute wash buffer (10x) 1 : 10 with deionized water before use.

Example: 10 mL wash buffer (10x) + 90 mL deionized water.

Dilute concentrated HRP conjugate (101x) 1 : 101 with sample diluent before use.

Example: 100 µL HRP conjugate (101x) + 10 mL sample diluent.

Prepare 1.0 mL of conjugate solution for each test strip. A fully used microplate requires 12.0 mL of conjugate solution. Prepare the conjugate solution at least 15 minutes before use. The diluted conjugate solution is stable for 1 day at room temperature.

Assay Procedure

One test kit (1x 96 wells) allows the quantitative determination of BSA in up to 41 samples when samples, standards and control are run in duplicate.

1. Allow test reagents and required number of wells to reach room temperature (RT). Shake reagents gently before use. Avoid foaming.
2. Pipette 100 µL **diluted sample**
 - 100 µL **STD 1 - 6** standards 1 - 6
 - 100 µL **CONTROL** control each.
3. Cover the plate and incubate for 60 min at RT.
4. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap dry onto absorbent paper if necessary.
5. Add 100 µL **diluted HRP conjugate** per well.
6. Cover the plate and incubate for 60 min at RT.
7. Decant, then wash each well 5x with 300 µL diluted wash buffer. Tap dry onto absorbent paper if necessary.
8. Add 100 µL **SUBSTR** substrate per well.
9. Incubate for 15 min at RT **protected from light**.
10. Add 100 µL **STOP** stop solution per well, mix gently.
11. 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 BSA concentrations (x-axis).

The 4-parameter regression model is recommended to create the reference curve.

The absorbance of an unknown sample is converted into a BSA concentration [ng/mL] by extrapolating from the reference curve. Samples with higher BSA concentrations than standard STD 1 (500 ng/mL) should be retested after further dilution. In case of pre-dilution of the samples, multiply the obtained concentration by the dilution factor.

Low concentrated samples alternatively may be tested in Serazym® Bovine Serum Albumin (BSA) sensitive (order no. E-108) with a measuring range of 2.5 ng/mL - 50 ng/mL.

Interpretation of Results

Test Validation

The test run is valid, if:

- mean absorbance $OD_{450/620nm}$ of standard STD 1 (500 ng/mL) ≥ 1.50 ,
- mean absorbance $OD_{450/620nm}$ of standard STD 6 (15 ng/mL) ≤ 0.50 and
- control is determined between 150 ng/mL and 250 ng/mL.

If one of the above-mentioned quality criteria is not met, test should be repeated strictly following the Instructions for Use (sample, wash buffer and conjugate dilution, incubation times and temperatures, wash steps, 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 an 8-fold determination in one test run:

Sample	Intra-assay coefficient of variation	
	Concentration [ng/mL]	CV [%]
1	499	9.3
2	291	7.5
3	123	5.6
4	101	4.7
5	50.3	9.2
6	24.7	9.9

The inter-assay coefficient of variation in Serazym® Bovine Serum Albumin was determined by a 2-fold determination in 18 individual test runs:

Sample	Inter-assay coefficient of variation	
	Concentration [ng/mL]	CV [%]
1	419	10.0
2	313	7.6
3	195	8.3
4	97.0	5.9
5	51.0	7.4
6	28.9	9.1

Cross-reactivity

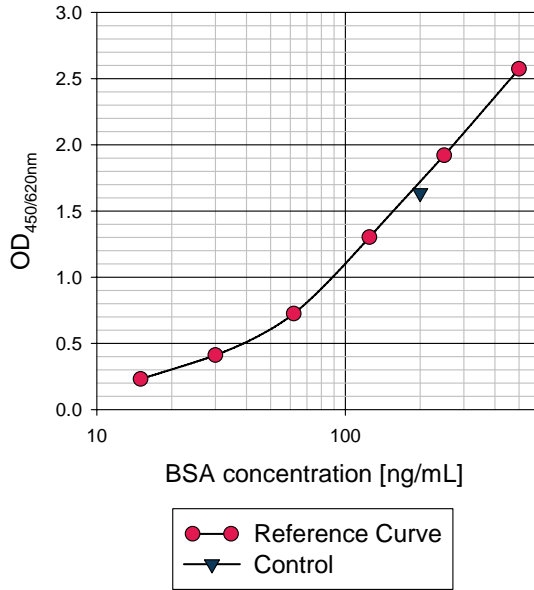
Human serum albumin, mouse serum albumin, rabbit serum albumin and ovalbumin were tested in Serazym® Bovine Serum Albumin. No cross-reactivity was detected within the range of the reference curve of Serazym® Bovine Serum Albumin.

Detection capability

The detection capabilities, including the limit of blank (LoB), limit of detection (LoD) and limit of quantification (LoQ), were determined in accordance with the CLSI guideline EP17-A2 for Serazym® Bovine Serum Albumin. The LoB, defined as the highest measurement result likely to be observed for a blank sample, was recorded as LoB = 0,69 ng/mL. An LoD of 1.45 ng/mL was established. The LoQ is defined as the lowest amount of measurand that can be quantitatively determined with an accuracy of less than 15 %. The LoQ for the Serazym® Bovine Serum Albumin was determined to be 1.97 ng/mL.

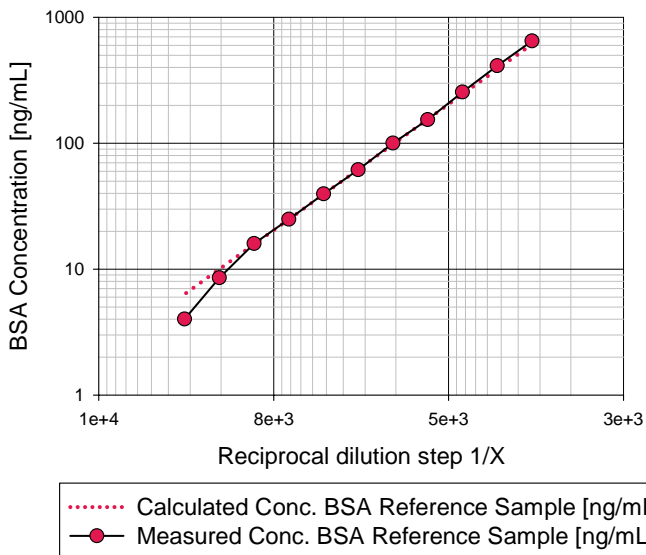
Reference Curve

A typical reference curve of Serazym® Bovine Serum Albumin is shown below:



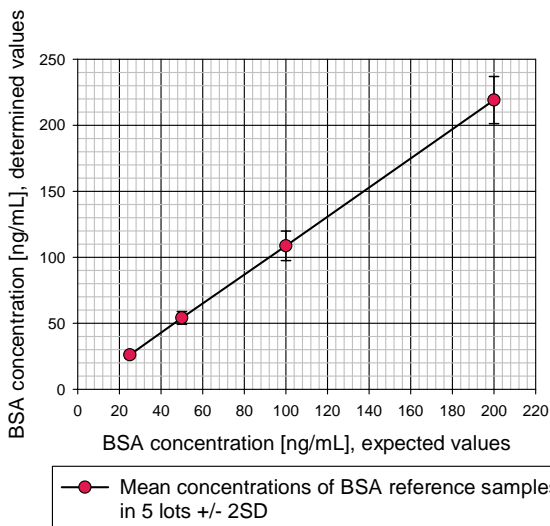
Linearity / Linear range

The linear range was determined in accordance with the CLSI guideline EP06-A for Serazym[®] Bovine Serum Albumin. To determine the concentration range for which the measurement signal is directly proportional to the analyte concentration in the sample, a BSA reference sample was diluted in stages into the linear measurement range, and the deviation from the theoretical dilution stage was determined. A linear relationship was confirmed between the theoretical concentration of BSA and the measured concentration of BSA in the STD 1 -6 range (500 ng/mL to 15 ng/mL) in Serazym[®] Bovine Serum Albumin.



Recovery

To determine recovery, BSA reference samples with theoretical concentrations of 200 ng/mL, 100 ng/mL, 50 ng/mL and 25 ng/mL were tested in five different lots. The lot-specific standards and reference samples were examined in quadruplicate. A reference curve was created using the mean absorbance value of each standard concentration. This curve was then used to calculate the concentrations of the different reference samples. The concentrations determined for the reference samples in the five different lots were plotted against their theoretical concentrations. The error bars show the twofold standard deviation ($\pm 2SD$).

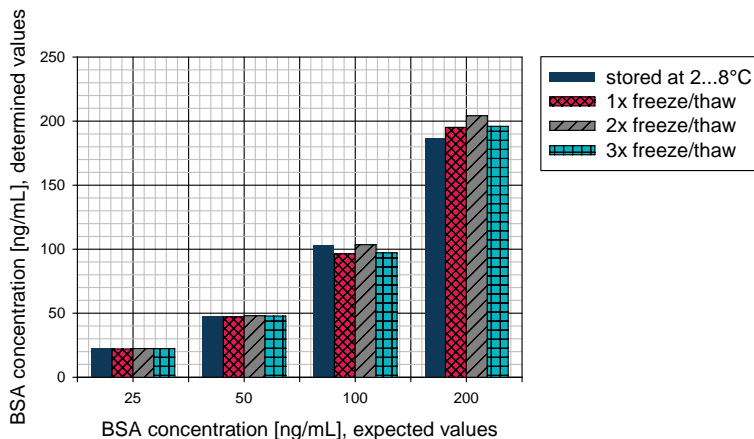


Expected BSA concentration [ng/mL]	Determined BSA concentration [ng/mL]	Recovery [%]
200.0	219.1	109.6
100.0	108.7	108.7
50.0	54.1	108.1
25.0	26.1	104.2

The recovery of BSA reference samples in five different lots has been determined in the range of 80–120% according to ICH guidelines.

Impact of Freezing and Thawing

BSA reference samples with concentrations of 200 ng/mL, 100 ng/mL, 50 ng/mL and 25 ng/mL were split into four aliquots. One aliquot was stored at 2...8 °C. The remaining three aliquots were frozen and thawed once, twice or three times. After three days, all samples were tested in triplicates in one test run. All samples were determined in the range of $\pm 2SD$ regardless of the number of freeze-thaw-cycles.



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
2026-01	Entire document	Update of Performance Characteristics Layout and editorial changes