Purity check

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Diagnostica GmbH

Serazym® Bovine Serum Albumin sensitive

Enzyme immunoassy for quantitative detection of bovine serum albumin (BSA)

- ▲ simultanous one-step assay
- ▲ short incubation times
- ▲ ready-to-use standards and control
- ▲ quantitative results (ng/ml)

Introduction

The *Serazym*® Bovine Serum Albumin sensitive ELISA enables the fast and sensitive quantification of bovine serum albumin (BSA) in vaccines and other biological fluids.

Principle of the *Serazym*® ELISA

The *Serazym*® Bovine Serum Albumin ELISA is a direct one–step two-site enzyme immunoassay using immobilized polyclonal antibodies to BSA and horseradish peroxidase labelled anti-BSA antibodies as detection system.

Test kit components

- 96-well microtitration plate
- 50 ml wash buffer, 10fold concentrated
- 70 ml sample diluent
- 7 x 1.0 ml BSA standards, ready-to-use
- 1.0 ml BSA control sample, ready-to-use
- 15 ml anti-BSA-HRP-conjugate, ready-to-use
- 15 ml TMB-/substrate solution, ready-to-use
- 15 ml stop solution

- **Attention: The *Serazym*® Bovine Serum Albumin sensitive ELISA is a very sensitive assay. It is recommended to use disposable reagent containers for pipetting the components. Make sure that the glassware and plastic material used for buffer preparation and reagent handling are absolutely free of BSA.**

Test procedure

- add 100 µl of the ready-to-use anti-BSA-HRP-conjugate into the intended wells
- add 100 µl of the diluted samples and of the ready-to-use standards and the control into the intended wells and shake carefully
- incubate 60 min at 20…25 °C
- wash wells 5 x
- add 100 µl of TMB-/substrate solution to every well
- incubate 15 min at 20…25 °C protected from light
- add 100 µl of stop solution to every well
- read absorbances at 450/620 nm

Quantification

Create a calibration curve using the absorbances of the standards with BSA concentrations in the range from 2.5 ng/ml to 50 ng/ml.
Determine the BSA concentrations of the samples by referring their absorbances to the corresponding concentrations of the calibration curve. Alternatively create a reference curve by including the sample diluent as standard S 8 with a concentration of 0.1ng/ml.
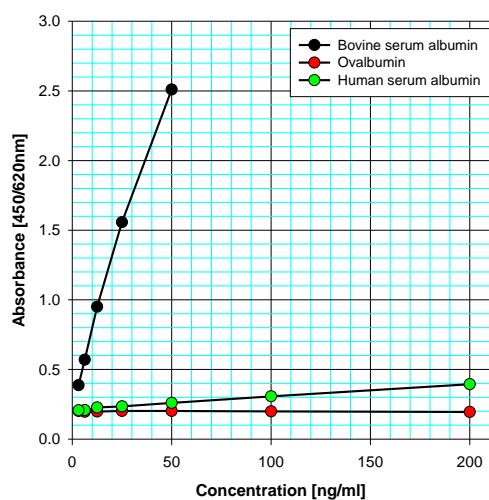
Validation

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Standard S 7	Absorbance	< 0.5
Standard S 1	Absorbance	> 1.5
Control sample	Concentration	20 - 30 ng/ml

Typical calibration curve
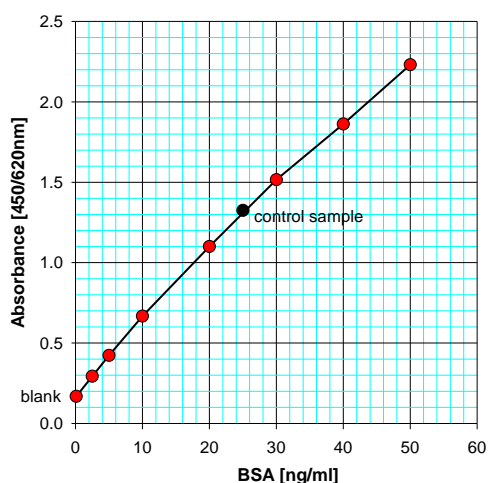
Cross reactivity

Intra-assay coefficient of variation (8-fold determination)

sample	Mean absorbance	Standard deviaton	Coefficient of variation [%]
1	2.437	0.033	1.43
2	2.060	0.035	1.79
3	1.670	0.050	3.18
4	1.243	0.051	4.41
5	0.737	0.029	4.24
6	0.473	0.013	2.86
7	0.322	0.002	0.51
8	1.494	0.037	2.61

sample	BSA concentration [ng/ml]	Standard deviaton	Coefficient of variation [%]
1	50.1	0.924	1.97
2	39.8	0.905	2.43
3	30.0	1.197	4.26
4	20.2	1.122	5.93
5	9.9	0.561	6.08
6	5.0	0.221	4.69
7	2.5	0.025	1.06
8	25.9	0.843	3.48

Linearity

r = 0.999
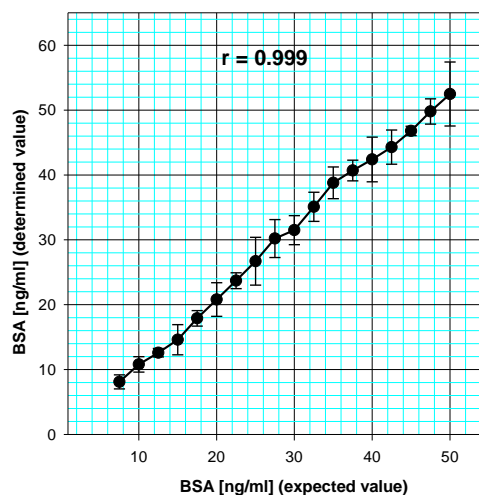
Inter-assay coefficient of variation (8 different test runs)

sample	Mean absorbance	Standard deviaton	Coefficient of variation [%]
1	2.087	0.099	5.07
2	1.205	0.067	5.93
3	0.715	0.037	5.50
4	0.466	0.027	6.74

sample	BSA concentration [ng/ml]	Standard deviaton	Coefficient of variation [%]
1	50.9	1.701	3.57
2	24.2	0.750	3.31
3	12.0	0.297	2.64
4	6.3	0.299	5.06

High Dosis Hook Effect