Porcine Soluble protein-100B (S-100B)ELISA Kit

Catalog No. CSB-E14063p

(96 tests)

- This immunoassay kit allows for the in vitro quantitative determination of **porcine S-100B** concentrations in **serum, plasma** and other biological fluids.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

S-100B is a member of the S100 family of proteins containing 2 EF-hand calcium binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21; however, this gene is located at 21q22.3. This protein may function in neurite extension, proliferation of melanoma cells, stimulation of Ca2+ fluxes, inhibition of PKC mediated phosphorylation, astrocytosis and axonal proliferation, and inhibition of microtubule assembly. Chromosomal rearrangements and altered expression of this gene have been implicated in several neurological, neoplastic, and other types of diseases, including Alzheimer's disease, Down's syndrome, epilepsy, amyotrophic lateral sclerosis, melanoma, and type I diabetes.

PRINCIPLE OF THE ASSAY

This assay employs the direct competitive inhibition enzyme immunoassay technique. An antibody specific for S-100B has been pre-coated onto a microplate. Add S-100B (Standards or samples) to the well, and then add Biotin-conjugated S-100B. A competitive inhibition reaction is launched between S-100B (Standards or samples) and Biotin conjugated S-100B with the S-100B antibody. Then add the HRP-avidin to each well. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of S-100B bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.625 ng/ml-10 ng/ml. The standard curve concentrations used for the ELISA's were 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml.

SPECIFICITY

This assay recognizes S-100B. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of S-100B is typically less than 0.312 ng/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	**Quantity**
Assay plate (96 tests)	1
Standard	5 x 1 ml
Conjugate	1 x 6 ml
HRP-Avidin	1 x 6 ml
Wash Buffer	1 x15 ml (20×concentrate)
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml

Standard	S1	S2	S3	S4	S5
Concentration（ng/ml）	0.625	1.25	2.5	5	10

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer..

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.

- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Set a Blank well without any solutions.
2. Add 50µl of **Standard** or **Sample** to per well.
3. Add 50µl of **Conjugate** to each well (not to the Blank!).
4. Cover with the adhesive strip. Incubate for 60 minutes at 37° C.
5. Aspirate each well and wash, repeating the process for a total of three to five washes. Wash by filling each well with Wash Buffer (about 200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 50µl of **HRP-avidin** to each well. Cover with the adhesive strip. Incubate for 30 minutes at 37°C.
7. Aspirate each well and wash as before.
8. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and divide the average zero standard optical density. Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the absorbance ratio for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the S-100B concentrations versus the ratio and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.