DRG® S100B (EIA-5169)

USA: RUO

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

Immunoenzymatic colorimetric method for determination of S100B concentration in human serum and plasma.

S100B ELISA kit is intended for laboratory use only.

2 PRINCIPLE

The S100B ELISA TEST is based on binding of S100B by two antibodies, one immobilized on microwell plates, and the other one conjugates with horseradish peroxidase (HRP).

The assay is on two steps binding procedure and after every incubation step, the bound/free separation is performed by a simple solid-phase washing, then the substrate solution (TMB) is added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. The S100B concentration in the sample is calculated based on a series of standard.
The color intensity is proportional to the S100B concentration in the sample.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. S100B **Standards** (6 vials, lyophilized)
   STD0 - STD5
2. **Controls** (2 vials, lyophilized)
   Negative Control
   Positive Control
3. **Conjugate Buffer** (1 vial, 20 mL)
   Tris buffer; BSA 10 g/L, Tween 0.05%
4. **Conjugate** (1 vial, 1 mL)
   Anti-S100B-HRP conjugate
5. **Assay Buffer** (1 vial, 12 mL)
   Tris buffer; BSA 10 g/L, stabilizing reagent
6. **Coated microplate** (1 breakable microplate)
   Anti-S100B adsorbed on microplate
7. **TMB-Substrate** (1 vial, 15 mL)
   H_2O_2-TMB 0.25 g/L (avoid any skin contact)
8. **Stop Solution** (1 vial, 15 mL)
   Sulphuric acid 0.15 mol/L (avoid any skin contact)

9. **50x Conc. Wash Solution** (1 vial, 20 mL)
NaCl 45g/L, Tween 20 55g/L

3.2 Necessary reagents not supplied with the kit

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader(450 nm)

3.4 Notes

Store all reagents between +2°C - 8°C in the dark.

Open the bag of reagent 6 (Coated Microplate) only when it is at room temperature and close it immediately after use.

Do not remove the adhesive sheets on the strips unutilized

4 PRECAUTIONS

The reagents contain Merthiolate Sodium as preservative.
Maximum precision is required for reconstitution and dispensation of the reagents.
All the reagents have a lot to lot consistency; do not mix various lot numbers kits components within a test.
This method allows the determination of S100B from 10 to 5000 pg/mL.
The calibrator concentrations are lot specific and are reported on the vial labels.
Do not use heavily hemolysed samples
Avoid the exposure of reagent TMB/H_2O_2 to direct sunlight, metals or oxidants.

5 PROCEDURE

5.1 Preparation of the sample

The S100B determination can be carried out in human serum or plasma. Do not use hemolyzed samples.

Samples can be stored at +2°C - 8°C for 1 day; for long periods store at -20°C. Avoid repeated freeze-thaw cycles. Do not leave the samples at room temperature (22 °C – 28 °C) for long period.

For sample with concentration higher than 5 ng/mL dilute the sample with Assay buffer

5.2 Preparation of the Standards and Controls

Reconstitute standards and controls with 1 mL of distilled water before use;
once reconstituted they are stable for 4 weeks at 2°C – 8°C and about six month if stored at -20°C.

It is advised to divide the content in aliquots and store them at -20°C.

The values of standard concentrations are reported on vial labels.

Avoid repeated freeze-thaw cycles and long time exposure at room temperature (22 °C – 28 °C).

5.3 Preparation of the Conjugate

Prepare 2 hours before use.

Add 50 µL Conjugate (reagent 4) to 1.0 mL of Conjugate Buffer (reagent 3).

(The quantity of diluted conjugate is proportional to the number of tests).

Mix gently for 5 minutes, with rotating mixer.

Stable for 3 hours at room temperature (22 °C – 28 °C).

5.4 Preparation of the wash solution

Dilute 10 mL of Wash Solution Concentrate (50X) with 490 mL of distilled or deionized; for different volumes keep dilution ratio. Store at room temperature (22 °C – 28 °C) until the expiry date written on the wash solution concentrate label.

5.5 Procedure

As it is necessary to perform the determination in duplicate, prepare two wells for each point of the standard curve (S_0-S_5), two for each Control, two for each sample, one for Blank.

Pipette:

Reagent	Standard	Sample / Control	Blank
Standard S0-S5, Controls	50 µL		
Sample / Control		50 µL	
Assay Buffer	50 µL	50 µL	
Incubate 2 hours at room temperature (22 °C – 28 °C). Remove the contents from each well, wash the wells six times with 300 µL of diluted wash solution			
Diluted Conjugate	100 µL	100 µL	
Incubate 1 h at room temperature (22 °C – 28 °C). Remove the content from each well, wash the wells six times with 300 µL of diluted wash solution			
TMB-Substrate	100 µL	100 µL	100 µL
Incubate 30 minutes at room temperature (22 °C – 28 °C), in the dark.			
Stop solution	100 µL	100 µL	100 µL
Shake gently the microplate. Read the absorbance (E) at 450 nm against Blank			

6 RESULTS

6.1 Mean Absorbance

Calculate the mean of the absorbencies (Em) for each point of the standard curve and of each sample.

6.2 Standard Curve

Plot the values of absorbance of the standards (S_0 –S_5) against concentration. Draw the best-fit curve through the plotted points (e.g.: Cubic spline or Four Parameter Logistic).

6.3 Calculation of Results

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations in pg/mL.

7 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations

8 TROUBLESHOOTING

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

no conjugate pipetted
contamination of conjugates and/or of substrate
errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

incorrect conjugate (e.g. not from original kit)
incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

incorrect conjugate (e.g. not from original kit)
incubation time too long, incubation temperature too high
water quality for wash buffer insufficient (low grade of deionization)
insufficient washing (conjugates not properly removed)

Unexplainable outliers

contamination of pipettes, tips or containers -insufficient washing (conjugates not properly removed)

too high within-run CV%

reagents and/or strips not pre-warmed to Room Temperature prior to use
plate washer is not washing correctly (suggestion: clean washer head)

too high between-run CV %

incubation conditions not constant (time, temperature)

controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
person-related variation

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