Plea...
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/H₂O₂ 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 (S₀-S₅), two for each Control, two for each sample, one for Blank.

<table>
<thead>
<tr>
<th>Pipette:</th>
<th>Standard</th>
<th>Sample / Control</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>Standard</td>
<td>Sample / Control</td>
<td>Blank</td>
</tr>
<tr>
<td>Standard S₀-S₅, Controls</td>
<td>50 μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample / Control</td>
<td>50 μL</td>
<td>50 μL</td>
<td></td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>50 μL</td>
<td>50 μL</td>
<td></td>
</tr>
</tbody>
</table>

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 (S₀ – S₅) 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)

correct variation

BIBLIOGRAPHY