DRG® S100B (Human) ELISA (EIA-4555)

Revised 20 Nov. 2010 rm (Vers. 9.1) **RUO**

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

The Human S100B ELISA is a sandwich enzyme immunoassay for measurement of human S100B.

Features

- **It is intended for research use only.**
- The total assay time is less than 5 hours.
- The kit measures S100B protein in serum, heparin plasma or cerebrospinal fluid.
- Assay format is 96 wells.
- Quality Controls are human serum based. Animal serum is used for Master Standard and for Dilution Buffer preparation.
- Standard is native protein based.
- Components of the kit are provided ready to use, concentrated or lyophilized.

2 STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3 TEST PRINCIPLE

In the Human S100B ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-cow S100B antibody. After 120 minutes incubation and washing, biotin-labelled monoclonal anti-human S100B antibody is added to the wells and incubated for 60 minutes with captured S100B. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of S100B. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

4 PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- This kit contains components of animal origin. These materials should be handled as potentially infectious.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

5 TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

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6 REAGENT SUPPLIED

1. Kit Components	2. State	3. Quantity
4. Antibody Coated Microtiter Strips	5. ready to use	6. 96 wells
7. Biotin Labelled Antibody Conc. (100x)	8. concentrated	9. 0.15 ml
10. Streptavidin-HRP Conjugate	11. ready to use	12. 13 ml
13. Master Standard	14. lyophilized	15. 1 vial
16. Quality Control HIGH	17. lyophilized	18. 1 vial
19. Quality Control LOW	20. lyophilized	21. 1 vial
22. Biotin-Ab Diluent	23. ready to use	24. 13 ml
25. Dilution Buffer	26. ready to use	27. 20 ml
28. Wash Solution Conc. (10x)	29. concentrated	30. 100 ml
31. Substrate Solution	32. ready to use	33. 13 ml
34. Stop Solution	35. ready to use	36. 13 ml
37. Product Data Sheet + Certificate of Analysis	38.	39. 1 pc

7 MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- Software package facilitating data generation and analysis (optional)

8 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

8.1 Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Biotin-Ab Diluent

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

8.2 Assay reagents supplied concentrated or lyophilized:

8.2.1 Human S100B Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard according to the Certificate of Analysis to prepare standard stock solution just prior to the assay. Let it dissolve for 25-30 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human S100B in the stock solution is **4000 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

40. Volume of Standard	41. Dilution Buffer	42. Concentration
43. Stock	44. -	45. 4000 pg/ml
46. 300 µl of stock	47. 300 µl	48. 2000 pg/ml
49. 300 µl of 2000 pg/ml	50. 300 µl	51. 1000 pg/ml
52. 300 µl of 1000 pg/ml	53. 300 µl	54. 500 pg/ml
55. 200 µl of 500 pg/ml	56. 300 µl	57. 200 pg/ml
58. 300 µl of 200 pg/ml	59. 300 µl	60. 100 pg/ml
61. 300 µl of 100 pg/ml	62. 300 µl	63. 50 pg/ml

Dilute each concentration of standard 4x with Dilution Buffer prior to the assay, e.g. 40 µl of standard + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of standard + 180 µl of Dilution Buffer for duplicates. Mix well (not to foam).

Stability and storage:

Standard stock solutions (4000 - 50 pg/ml) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions.

8.2.2 Quality Controls High, Low

Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution and for current Quality Controls concentrations!!!

Reconstitute each Quality Control (High and Low) with **distilled water** just prior to the assay. Let it dissolve for 25-30 minutes with occasional gentle shaking (not to foam).

Dilute Quality Controls prior to the assay 4x with Dilution Buffer, e.g. 40 µl of Quality Control + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of Quality Control + 180 µl of Dilution Buffer for duplicates

Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Controls.

8.2.3 Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part of Biotin Labelled Antibody Concentrate (100x) to 99 parts of Biotin-Ab Diluent.

Example (for 1 strip, i.e. 8 wells): 10 µl of Biotin Labelled Antibody Conc. (100x) + 990 µl Biotin-Ab Diluent.

Stability and storage:

Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.

Do not store diluted Biotin Labelled Antibody working solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.
Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

9 PREPARATION OF SAMPLES

The kit measures S100B in serum, heparin plasma and cerebrospinal fluid samples.

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 4x with Dilution Buffer just prior to the assay, e.g. 40 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of sample + 180 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

See Chapter 13 for stability of cerebrospinal fluid sample when stored at 2-8°C and effect of freezing/thawing on the concentration of S100B protein.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

10 ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate at room temperature (ca. 25°C) for **120 minutes**, shaking at ca. 300rpm on an orbital microplate shaker.
3. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells **5-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **15 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Substract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

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Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine S100B concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

64.	65. strip 1+2	66. strip 3+4	67. strip 5+6	68. strip 7+8	69. strip 9+10	70. strip 11+12
71. A	**72. Standard 2000**	**73. Blank**	74. Sample 8	75. Sample 16	76. Sample 24	77. Sample 32
78. B	**79. Standard 1000**	80. Sample 1	81. Sample 9	82. Sample 17	83. Sample 25	84. Sample 33
85. C	**86. Standard 500**	87. Sample 2	88. Sample 10	89. Sample 18	90. Sample 26	91. Sample 34
92. D	**93. Standard 200**	94. Sample 3	95. Sample 11	96. Sample 19	97. Sample 27	98. Sample 35
99. E	**100. Standard 100**	101. Sample 4	102. Sample 12	103. Sample 20	104. Sample 28	105. Sample 36
106. F	**107. Standard 50**	108. Sample 5	109. Sample 13	110. Sample 21	111. Sample 29	112. Sample 37
113. G	**114. QC High**	115. Sample 6	116. Sample 14	117. Sample 22	118. Sample 30	119. Sample 38
120. H	**121. QC Low**	122. Sample 7	123. Sample 15	124. Sample 23	125. Sample 31	126. Sample 39

Figure 1: Example of a work sheet.

11 CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of S100B pg/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

Samples, Quality Controls and Standards are all diluted 4x prior to analysis, so there is no need to take this dilution factor into account.

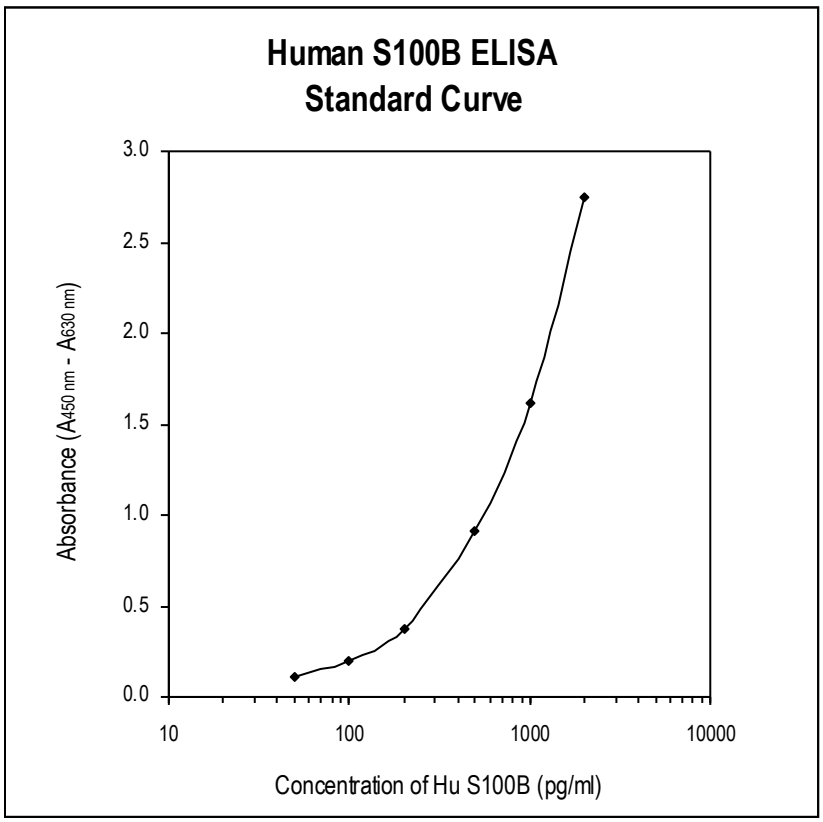

Figure 2: Typical Standard Curve for Human S100B ELISA.

12 METHOD COMPARISON

The Human S100B ELISA has not been compared to the other commercial immunoassays.

13 TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

14 REFERENCES / LITERATURE

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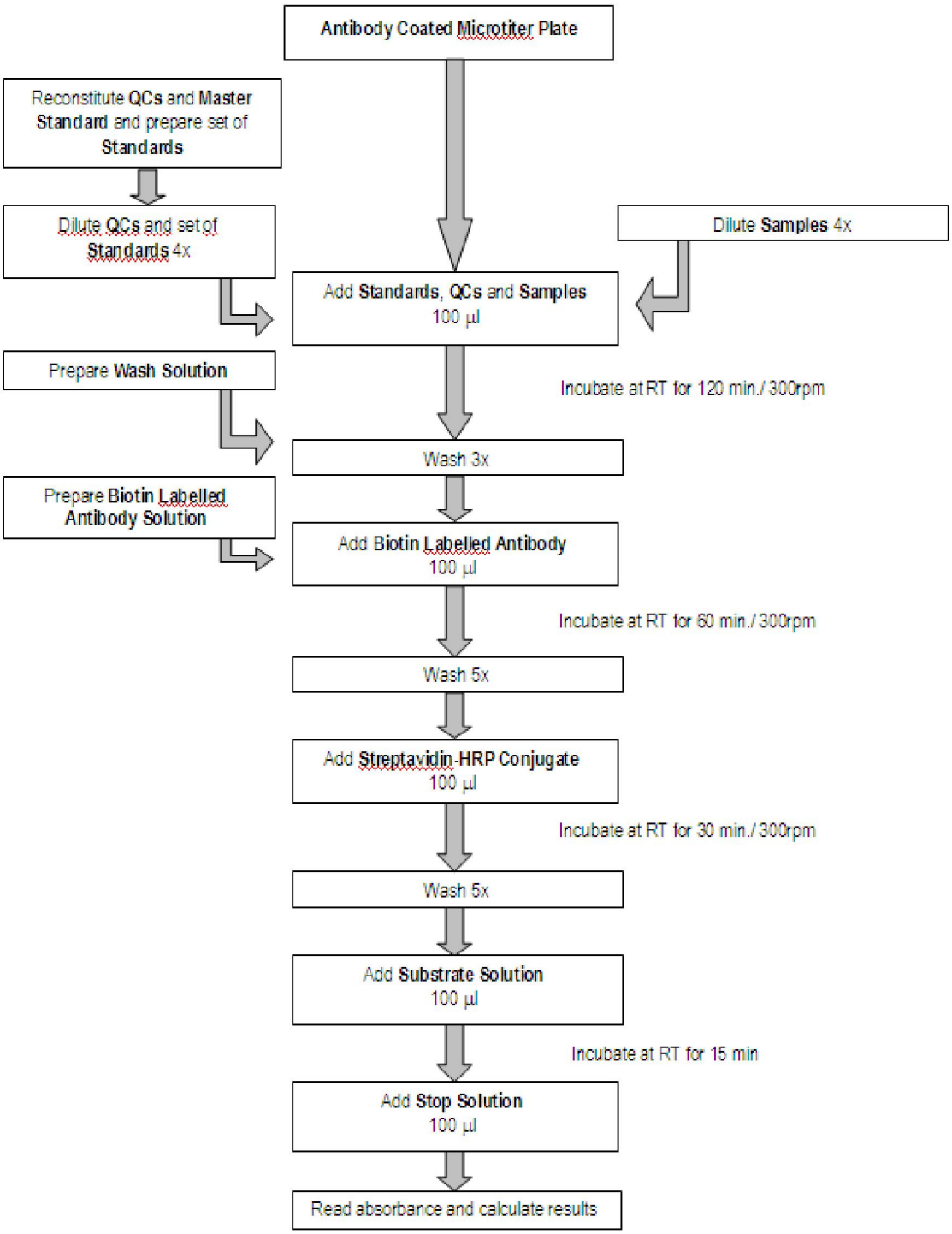
15 ASSAY PROCEDURE SUMMARY



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