



CanAg S100A1B EIA

Prod. No. 706-85

Instructions for use
2006-12

Enzyme immunometric assay kit
For 96 determinations

For Research Use Only-Not for use in diagnostic procedures

INTENDED USE

The CanAg S100A1B EIA kit is intended for the quantitative determination of S100A1B in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

S100 is a 20 kDa protein belonging to the S100/calmodulin/troponin C superfamily of EF-hand calcium-binding proteins. S100 was originally isolated from human brain, and considered a glial-cell specific protein (1). Today, 20 monomers of the S100 family have been identified based on structural and functional similarities (2, 3). Most of the S100 proteins are highly conserved between species and exist as dimers, expressed in a cell-specific manner (2). One of the S100 monomers, designated S100B (4) is found as homo- (BB) and hetero-dimers (A1B), predominantly in nervous system glial cells but also in certain peripheral cells e.g. melanocytes, adipocytes, and chondrocytes (5). In the nervous system, astrocytes and oligodendrocytes express both isoforms of S100B while Schwann cells have been reported to specifically express S100BB (5). Both S100A1B and S100BB are also present in malignant tissues, most notably in melanoma and to a lesser extent in glioma, thyroid cell carcinoma and renal cell carcinoma (2).

PRINCIPLE OF THE TEST

The CanAg S100A1B EIA is a solid-phase, two-step, non-competitive immunoassay based on two mouse monoclonal antibodies specific for two different epitopes specifically expressed in S100A1B. The assay thus determines S100A1B with very low cross-reactivity with S100BB or other forms of S100.

Calibrators and unknown samples are incubated together with biotinylated Anti-S100 monoclonal antibody (MAb) S23 in Streptavidin coated microtiter strips. S100A1B present in calibrators or samples is

adsorbed to the Streptavidin coated micro wells by the biotinylated Anti-S100 MAb during the incubation. The strips are then washed and incubated with horseradish peroxidase (HRP) labelled Anti-S100A1B MAb S35. After washing, buffered Substrate/ Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportional to the amount of S100A1B present in the samples.

The colour intensity is determined in a micro plate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The S100A1B concentrations of unknown samples are then read from the calibration curve.

REAGENTS

- Each CanAg S100A1B EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8° C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first opening
MICROPLA Streptavidin Microplate	1 Plate	2–8°C until expiry date stated on the plate
12 x 8 breakable wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.		

S100A1B Calibrators	6 vials, lyophilised	4 weeks at 2–8° C 3 months at –30° C or below
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CAL	S100A1B	A
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1 x 1 mL

CAL	S100A1B	B
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1 x 1 mL

CAL	S100A1B	C
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1 x 1 mL

CAL	S100A1B	D
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1 x 1 mL

Component	Quantity	Storage and stability after first opening
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CAL	S100A1B	E
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1 x 1 mL

CAL	S100A1B	F
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1 x 1 mL

The lyophilised calibrators contain bovine S100A1B in a protein matrix with 0.02% NaN₃ as preservative. To be reconstituted with water before use. **NOTE:** The exact S100A1B concentration is lot specific and is indicated on the label of each vial.

BIOTIN	Anti-S100
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Biotin Anti-S100

1 x 15 mL

2–8° C until expiry date
stated on the vial

Biotin Anti-S100 monoclonal antibody from mouse, approximately 2 µg/mL. Contains phosphate buffered saline (pH 7.2) with CaCl₂, bovine serum albumin, bovine immunoglobulin, blocking agents, Tween 20, an inert blue dye and 0.01% methyl-isothiazolone (MIT) as preservative. Ready for use.

CONJ	Anti-S100A1B
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Tracer, HRP Anti-S100A1B

1 x 0.75 mL

2–8° C until expiry date
stated on the vial

Stock solution of HRP Anti-S100A1B monoclonal antibody from mouse, approximately 20 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL	CONJ
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Tracer Diluent

1 x 15 mL

2–8°C until expiry date
stated on the vial

Phosphate buffered saline (pH 7.2) with bovine serum albumin, blocking agents, detergents, an inert blue dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

SUBS	TMB
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TMB HRP-Substrate

1 x 12 mL

2–8°C until expiry
date stated on the vial

Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

STOP

STOP Solution

1 x 15 mL

2–8°C until expiry
date stated on the vial

Contains 0.12 M hydrochloric acid. Ready for use.

Component	Quantity	Storage and stability after first opening
WASHBUF 25X		
Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with water 25 times before use.		

Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

- For professional use only.
- Please refer to the US Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all human specimens as potentially infectious.
- Reagents contain sodium azide (NaN₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
- Follow local guidelines for disposal of all waste material.

SPECIMEN COLLECTION AND HANDLING

The CanAg S100A1B EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8° C for 24 hours. For longer periods it is recommended to store the samples at –20°C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. Microplate shaker

Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 700-900/min.

2. Microplate wash device

Automatic plate wash capable of performing 1, 3 and 6 washing cycles, or a semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid. The Nunc Immuno-8 manual strip washer is recommended if an automatic microplate wash is not used.

3. Microplate spectrophotometer

With a wavelength of 620 nm and/or 405 nm, and an absorbance range of 0 to 3.0.

4. Precision pipettes

With disposable plastic tips to deliver microlitre and millilitre volumes. An 8-channel pipette or dispenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential.

5. Distilled or deionised water

For reconstitution of S100A1B Calibrators and for preparation of Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg S100A1B EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25°C) prior to use. The assay should only be performed at temperatures between 20–25°C to obtain accurate results. Frozen specimens should be brought to room temperature slowly and must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and unknown samples it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. A careful washing procedure of the strips is essential. Ensure that each well is filled up completely to the top edge and that the aspiration of the wells between and after the washing cycles is complete and the wells are dry. If there is liquid left in the wells, invert the plate and tap it carefully against absorbing paper.

Automatic strip washer: Follow the manufacturer's instructions for maintenance and wash the required number of wash cycles prior to and after each incubation step.

The aspiration/wash device should not be left standing with the Wash Solution for long periods as the needles may get clogged, giving poor liquid delivery and suction.

5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or dispenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate Solution.

Preparation of reagents	Stability of prepared reagent
S100A1B Calibrators	4 weeks at 2–8°C 3 months at –30° C or below
Add exactly 1.0 mL of distilled water to each vial and mix gently. Allow to stand for at least 15 minutes to reconstitute. NOTE: The concentration of the calibrators is stated on the labels and should be used for calculation of results.	
Wash Solution	2 weeks at 2–25°C in a sealed container
Pour the 50 mL Wash Concentrate into a clean container and dilute 25-fold by adding 1200 mL of distilled or deionised water to give a buffered Wash Solution.	
Tracer working solution	3 weeks at 2–8°C in a sealed container
Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-S100A1B with 1 mL of Tracer Diluent per strip (see table below):	

No. of Strips	Tracer, HRP Anti-S100A1B (µL)	Tracer Diluent (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of the Tracer working solution.

Alternative: Pour the content of the Tracer, HRP Anti-S100A1B into the vial of Tracer Diluent and mix gently. Make sure that all of the Tracer, HRP Anti-S100A1B is transferred to the vial of Tracer Diluent.

NOTE: The Tracer working solution is stable for 3 weeks at 2–8°C. Do not prepare Tracer working solution than will be used within this period and make sure that it is stored properly.

Assay procedure

Perform each determination in duplicate for calibrators and patient samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25°C) before use.

1. Start to prepare S100A1B Calibrators, Wash Solution and Tracer working solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 50 μ L of the S100A1B Calibrators (CAL A, B, C, D, E, F) and unknown samples (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E					
B	Cal A	Cal E					
C	Cal B	Cal F					
D	Cal B	Cal F					
E	Cal C	Unk1					
F	Cal C	Unk1					
G	Cal D	Unk2					
H	Cal D	Unk2					

4. Add 100 μ L of Biotin Anti-S100 to each well using a 100 μ L precision pipette (or an 8-channel 100 μ L precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.
5. Incubate the frame containing the strips for 2 hours (\pm 10 min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
6. After the first incubation aspirate and wash each strip 3 times using the wash procedure described in Procedural notes, item 4.
7. Add 100 μ L of Tracer working solution to each well. Use the same pipetting procedure as in item 4 above.
8. Incubate the frame for 1 hour (\pm 5 min) at room temperature (20–25°C) with constant shaking.
9. After the second incubation aspirate and wash each strip 6 times, using the wash procedure described in Procedural notes, item 4.
10. Add 100 μ L of TMB HRP-Substrate to each well using the same pipetting procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the addition to the first and last well should not exceed 5 min.
11. Incubate for 30 min (\pm 5 min) at room temperature with constant shaking. Avoid direct sunlight.
12. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

Option

If the laboratory does not have access to a microplate spectrophotometer capable of reading at 620 nm, the absorbance can be determined as follows:

Alt. 12. Add 100 µL of Stop Solution. Mix and read absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg S100A1B EIA measures concentrations between 30 and 3000 ng/L. If S100A1B concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human serum prior to analysis. **NOTE:** The serum used for dilution should also be measured in order to determine the endogenous S100A1B concentration (see “Calculation of results”).

Quality control

The use of internal control sera is advised to assure the day-to-day validity of results. It is recommended that the laboratory prepare its own serum pools with at least two levels (low and high) of S100A1B as controls.

Reference material

Since no common reference material is available for S100A1B, CanAg S100A1B Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer reader with built-in data calculation program is used, refer to the manual for the plate reader and create a program using the concentration stated on the labels of each of the S100A1B Calibrators.

For automatic calculation of S100A1B results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 ng/L.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 ng/L.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 ng/L.

NOTE: 4-parametric or linear regression should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each S100A1B calibrator against the corresponding S100A1B concentration (in ng/L), see figure below. The unknown S100A1B concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

If samples in an initial analysis give S100A1B levels higher than Calibrator F (circa 3000 ng /L) the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate

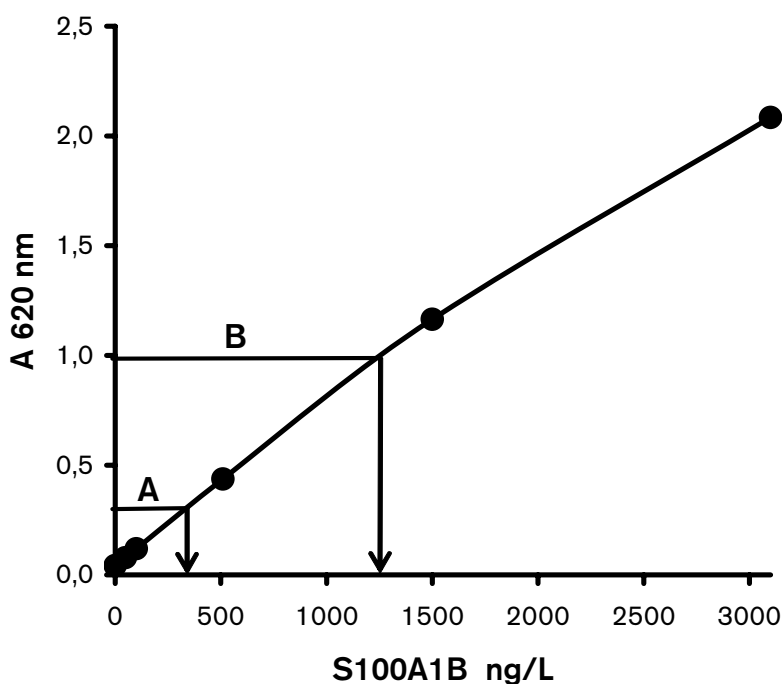
S100A1B concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the endogenous S100A1B concentration.

The S100A1B concentration of the undiluted sample is calculated as:

$$\text{Dilution 1/10:} \quad 10 \times ([S100]_{\text{Diluted sample}} - (0.9 \times [S100]_{\text{Normal serum}}))$$

Example of results

Specimen			Calibrator Values	Mean abs value (A)	S100 ng/L
CAL	S100A1B	A	0 ng/L	0.038	
CAL	S100A1B	B	48 ng/L	0.075	
CAL	S100A1B	C	100 ng/L	0.114	
CAL	S100A1B	D	510 ng/L	0.203	
CAL	S100A1B	E	1500 ng/L	0.830	
CAL	S100A1B	F	3100 ng/L	2.101	
Specimen A				0.282	310
Specimen B				0.995	1258



Example (do not use this curve or table above to determine actual assay results).

LIMITATIONS OF THE PROCEDURE

S100A1B have not been evaluated for in vitro diagnostic use and the level of S100A1B should not be used as evidence for the presence or absence of disease.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

LITERATURE REFERENCES

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