



CanAg S100 EIA

**For Research Use Only.
Not for use in
diagnostic procedures.**

REF 708-85

Instructions for use. 2006-10

Enzyme immunometric assay kit
For 96 determinations

GB EXPLANATION OF SYMBOLS
DE BEDEUTUNG DER SYMBOLE
ES EXPLICACIÓN DE SÍMBOLOS
IT SIGNIFICATO DEI SIMBOLI
FR EXPLICATION DES SYMBOLES
DK SYMBOLFORKLARING
GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
SE SYMBOLFÖRKLARING



Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Holdbar til/Ημερομηνία λήξης/
Bäst före datum

LOT

Batch code/Chargenbezeichnung/
Codigo de lote/
Codice del lotto/Code du lot/
Lotnummer/Αριθμός Παρτίδας/
Lotnummer



Date of manufacture/
Herstellungsdatum/
Fecha de fabricación/
Data di fabbricazione/
Date de fabrication/
Produktionsdato/
Ημερομηνία Παραγωγής/
Tilverkningsdatum

REF

Catalogue number/Bestellnummer/
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Référence du catalogue/
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Produktnummer



Manufacturer/Hersteller/Fab-
ricante/Fabbricante/Fabricant/
Producent/τασκευαστής/
Tilverkare



Contains sufficient for <96> tests/
Ausreichend für "96" Ansätze/
Contenido suficiente
para <96> ensayos/
Contenuto sufficiente per "96" saggi/
Contenu suffisant pour "96" tests/
Indeholder tilstrækkeligt
til "96" test/
Περιεχόμενο επαρκές
για «96» εξετάσεις/
Innehåller tillräckligt till "96" tester



Temperature limitation/
Zulässiger Temperaturbereich/
Limite de temperatura/
Limiti di temperatura/
Limites de température/
Temperaturbegrensning/
Περιορισμοί θερμοκρασίας/
Temperaturgräns



Consult Instructions for Use/
Gebrauchsanweisung beachten/
Consulte las instrucciones de uso/
Consultare le istruzioni per l'uso/
Consulter les instructions d'utilisation/
Se brugsanvisning/
Συμβουλευτείτε τις οδηγίες
χρήσης/
Se bruksanvisning



Biological risks/Biogefährdung/
Riesgo biológico/Rischio biologico/
Risques biologiques/
Biologisk fare/Bιολογικοί κίνδυνοι/
Biologisk risk



Contents of kit/Inhalt/Contenido/
Contenido/Contenu/Indhold/
ανιδραστήρια/Kit innehåll



From mouse/der Maus/de ratón/
Murino/De souris/Mus/απτο ποντίκι/
Från mus



Human/Human/Humano/
Origine Humana/Humaine/Human
δείγματα αναφοράς/Human

INTENDED USE

The CanAg S100 EIA kit is intended for the quantitative determination of S100B (S100A1B + S100BB) in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

S100 is a 20 kDa protein belonging to the S100/calmodulin/troponin C super-family 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 exist as dimers and are expressed in a cell-specific manner. Two of the S100 monomers, designated S100A1 and S100B (4) are highly conserved between species and are found as homo- (BB) and heterodimers (A1B) in central nervous system glial cells and in certain peripheral cells eg. Schwann cells, melanocytes, adipocytes, and chondrocytes (5). 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 S100 EIA is a solid-phase, two-step, non-competitive immunoassay based on two mouse monoclonal antibodies specific for two different epitopes expressed in S100B. The assay determines both S100A1B and S100BB without cross-reactivity with other forms of S100. Calibrators and samples are incubated together with biotinylated Anti-S100B monoclonal antibody (MAb) S23 in Streptavidin coated microstrips. S100B present in calibrators or samples is adsorbed to the Streptavidin coated microwells by the biotinylated Anti-S100B MAb during the incubation. The strips are then washed and incubated with horseradish peroxidase (HRP) labelled Anti-S100B MAb S53. 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 S100B present in the samples.

The colour intensity is determined in a microplate 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 S100B concentrations of samples are then read from the calibration curve.

REAGENTS

- Each CanAg S100 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
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MICROPLA

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

Component	Quantity	Storage and stability after first opening			
S100 Calibrators	6 vials, lyophilized	4 weeks at 2–8° C 3 months at –30° C or below			
<table border="1"><tr><td>CAL</td><td>S100</td><td>A</td></tr></table>	CAL	S100	A	1 x 1 mL	
CAL	S100	A			
<table border="1"><tr><td>CAL</td><td>S100</td><td>B</td></tr></table>	CAL	S100	B	1 x 1 mL	
CAL	S100	B			
<table border="1"><tr><td>CAL</td><td>S100</td><td>C</td></tr></table>	CAL	S100	C	1 x 1 mL	
CAL	S100	C			
<table border="1"><tr><td>CAL</td><td>S100</td><td>D</td></tr></table>	CAL	S100	D	1 x 1 mL	
CAL	S100	D			
<table border="1"><tr><td>CAL</td><td>S100</td><td>E</td></tr></table>	CAL	S100	E	1 x 1 mL	
CAL	S100	E			
<table border="1"><tr><td>CAL</td><td>S100</td><td>F</td></tr></table>	CAL	S100	F	1 x 1 mL	
CAL	S100	F			

The lyophilized calibrators contain bovine S100B in a protein matrix with a non-azide preservative. To be reconstituted with water before use. **NOTE:** The exact S100B concentration is lot specific and is indicated on the label of each vial.

<table border="1"><tr><td>BIOTIN</td><td>Anti-S100</td></tr></table>	BIOTIN	Anti-S100		
BIOTIN	Anti-S100			
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.

<table border="1"><tr><td>CONJ</td><td>Anti-S100</td></tr></table>	CONJ	Anti-S100		
CONJ	Anti-S100			
Tracer, HRP Anti-S100	1 x 0.75 mL	2–8°C until expiry date stated on the vial		

Stock solution of HRP Anti-S100 monoclonal antibody from mouse, approximately 20 µg/mL. Contains preservatives. To be diluted with Tracer Diluent before use.

Component	Quantity	Storage and stability after first opening
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DIL	CONJ
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Tracer Diluent	1 x 15 mL	2–8°C until expiry date stated on the vial
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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
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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
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Contains 0.12 M hydrochloric acid. Ready for use.

WASHBUF	25X
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Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
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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 Research Use Only. Not for use in diagnostic procedures.

- 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 serum specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

SPECIMEN COLLECTION AND HANDLING

The CanAg S100 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 microplatewash 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 respenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential.

5. Distilled or deionized water

For reconstitution of S100 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 S100 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 specimens 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
S100 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
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Pour the 50 mL Wash Concentrate into a clean container and dilute 25- fold by adding 1200 mL of distilled or deionized water to give a buffered Wash Solution.

Tracer working solution	3 weeks at 2–8°C in a sealed container
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Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-S100 with 1 mL of Tracer Diluent per strip (see table below):

No. of Strips	Tracer, HRP Anti-S100 (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-S100 into the vial of Tracer Diluent and mix gently. Make sure that all of the Tracer, HRP Anti-S100 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 unknown 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 S100 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 µL of the S100 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	etc.				
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 µL of Biotin Anti-S100 to each well using a 100 µL precision pipette (or an 8-channel 100 µL 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 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 μ L 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 S100 EIA measures concentrations between 10 and 3500 ng/L. If S100B 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 S100B concentration (see “Calculation of results”).

Quality control

CanChek Tumor Marker Control Sera Levels 1 and 2 (available separately, REF 107-20) are recommended for validation of the assay series. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated.

Reference material

Since no common reference material is available for S100A1B or S100BB, CanAg S100 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 S100 Calibrators.

For automatic calculation of S100 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 S100 calibrator against the corresponding S100 concentration (in ng/L), see figure below. The unknown S100 concentrations can then be read from the calibration curve using the mean absorbance value of each specimen.

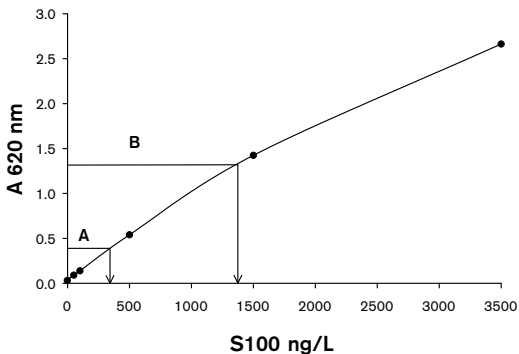
If samples in an initial analysis give S100 levels higher than Calibrator F (circa 3500 ng /L) the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate S100 concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the endogenous S100 concentration.

The S100 concentration of the undiluted sample is calculated as:

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

Example of results

Specimen			Calibrator values	Mean abs value (A)	S100 (ng/L)
CAL	S100	A	0 ng/L	0.041	
CAL	S100	B	50 ng/L	0.091	
CAL	S100	C	100 ng/L	0.139	
CAL	S100	D	500 ng/L	0.540	
CAL	S100	E	1500 ng/L	1.425	
CAL	S100	F	3500 ng/L	2.663	
Specimen A				0.352	305
Specimen B				1.377	1435



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

LIMITATIONS OF THE PROCEDURE

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

1. Moore BW (1965) A soluble protein characteristic of the nervous system. *Biochem Biophys Res Commun* 19:739-744.
2. Zimmer DB et al., (1995) The S100 protein family history, function and expression. *Brain Res Bull* 37:417-429.
3. Heizmann CW et al., (2002) S100 proteins: structure, functions and pathology. *Front Biosci* 7:1356-1368.
4. Schäfer BW et al. (1995) Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: rationale for a new nomenclature of the S100 calcium-binding protein family. *Genomics* 25:638-643.
5. Takahashi K et al., (1984) Immunohistochemical study on the distribution of α and β subunits of S-100 protein in human neoplasm and normal tissues. *Virchows Arch* 45:385-396.



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