



CanAg S100 EIA

REF 708-10

IVD



Instructions for use. 2003-11

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- DK** Kontakt venligst den danske distributør for gældende version af dansk brugsanvisning.
- GR** Παρακαλούμε όπως επικοινωνήσετε με τον προμηθευτή σας για την έγκυρη απόδοση στα Ελληνικά των οδηγιών χρήσης
- SE** Vänligen kontakta Er distributör för gällande version av bruksanvisning på svenska.

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

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/
Tillverkningsdatum



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

REF

Catalogue number/Bestellnummer/
 Número de catálogo/
 Numero di catalogo/
 Référence du catalogue/
 Katalognummer/
 Αριθμός καταλόγου/
 Produktnummer



Manufacturer/Hersteller/Fabricante/
 Fabbricante/Fabricant/
 Producent/τασκευαστής/
 Tillverkare



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

IVD

In Vitro Diagnostic Medical Device/
 In Vitro Diagnostikum/ Producto sanitario para diagnóstico in vitro/
 Dispositivo medico diagnostico in vitro/
 Dispositif médical de diagnostic in vitro/
 Medicinsk udstyr til in vitro-diagnostik/
 In Vitro diagnostický zdravotnický prostředek/
 In Vitro Διαγνωστικό
 Ιατροτεχνολογικό προϊόν/
 Endast för in vitro-diagnostik



Temperature limitation/
 Zulässiger Temperaturbereich/
 Limite de temperatura/
 Limiti di temperatura/
 Limites de température/
 Temperaturbegrænsning/
 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

CONT

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

ORIG MOU

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

ORIG BOV

Bovine/Rinder/Vaca/Vacca/
 Vache/Ko/αγελάδα/Från ko

WARNINGS AND PRECAUTIONS

GB

For in vitro diagnostic use

- For Professional Use Only
- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.
- Handle all patient specimens as potentially infectious.
- Reagents contain sodium azide (NaN_3) 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.

VORSICHTSMASSNAHMEN

DE

Nur zur in vitro-Diagnostik zu verwenden

- Nur für den professionellen Gebrauch
- Für die Sicherheit im Labor lesen Sie bitte Publikation: U.S. Department of Health and Human Services (Bethesda, MD, USA/ No. (CDC) 88-8395) oder kontaktieren Sie Ihre lokale/nationale Behörde.
- Behandeln Sie sämtliche Probe mit Vorsicht – Sie sind potentiell infektiös.
Chemikalien und Zubereitungen, die als Reststoffe anfallen, sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den abfallrechtlichen Gesetzen und Verordnungen des Bundes und der Länder. Die zuständige
- Die Reagenzien enthalten Natriumazid (NaN_3) als Konservierungsmittel. Natriumazid kann mit Blei- und Kupferleitungen reagieren und hochexplosive Metallazide bilden. Spülen Sie die Leitungen beim Wegschütten mit viel Wasser, um einer Azidbildung vorzubeugen.
- Behörde oder Abfallbeseitigungsunternehmen informieren über die Entsorgung von Sonderabfällen.

CUIDADOS Y PRECAUCIONES

ES

Para diagnóstico in vitro

- Solo para uso profesional
- Consultar la publicación del U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 o las normas locales o nacionales.
- Tratar todas las muestras de pacientes como potencialmente infecciosas.
- Los reactivos contienen azida sódica (NaN_3) como conservante. La azida sódica puede reaccionar con el plomo o el cobre de las tuberías, formando azidas metálicas muy explosivas. Al limpiar los reactivos, dejar correr gran cantidad de agua para evitar la formación de azidas.
- Todos los residuos se deben tirar cumpliendo las normas en vigor.

AVVERTENZE E PRECAUZIONI

IT

Per uso diagnostico in vitro

- Solamente per uso professionale
- Come riferimento si consiglia la pubblicazione No. (CDC) 88-8395 del US Department of Health and Human Service o qualsiasi altro regolamento locale o nazionale relativo alle Norme di Sicurezza da seguire nei Laboratori Diagnostici
- Maneggiare i campioni dei pazienti come potenzialmente infetti
- I reattivi contengono sodio azide (NaN_3) come conservante. Il sodio azide può reagire con piombo e rame formando azidi metallici altamente esplosivi. Quando i reattivi vengono scartati lavare con abbondante quantità di acqua per prevenire il rischio di reazione dell'azide
- Seguire le normative vigenti relative all'eliminazione del materiale usato

PRÉCAUTIONS D'EMPLOI ET MISE EN GARDE

FR

Pour un usage diagnostique *in vitro*

- Pour usage professionnel seulement.
- Prière de se référer à la Publication N° : (CDC) 88-8395 de l'U.S. Département of Health and Human Services (Bethesda, Md., USA) sur les procédures de sécurité dans les laboratoires ou toutes autres réglementations locales et nationales.
- Manipuler les échantillons de patients comme potentiellement infectieux.
- Réactifs contenant de l'Azide de Sodium (NaN₃) comme conservateur: l'Azide de Sodium peut réagir avec les tubes en plomb et en cuivre pour former des Azides de métaux hautement explosifs. Lors de l'élimination, répandre une grande quantité d'eau pour prévenir la formation des Azides.
- Suivre les réglementations locales pour l'élimination et le traitement de tous les déchets.

ADVARSLER OG FORHOLDSREGLER

DK

Til *in vitro* diagnostisk anvendelse

- Kun til professionel brug
- Der henvises til U.S. Department of Health and Human Services (de amerikanske sundhedsmyndigheder) (Bethesda, Md., USA) udgivelse nr. (CDC) 88-8395 vedrørende laboratoriesikkerhedsprocedurer eller andre lokale eller nationale forskrifter.
- Alle patientprøver skal behandles som potentielt smittefarlige.
- Reagenser indeholder natriumazid som præserveringsmiddel. Natriumazid kan danne eksplosive syrer i metalaflebe. Anvend korrekt affaldsprocedure.
- Følg lokale regler for afskaffelse af alt affald.

ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ ΚΑΙ ΠΡΟΦΥΛΑΞΕΙΣ

GR

Για *in vitro* διαγνωστική χρήση

- Για επαγγελματική χρήση, μόνο.
- Παρακαλούμαι όπως επικαλεστείτε τις οδηγίες ασφαλούς λειτουργίας των εργαστηρίων του Τμήματος Υγείας και Ανθρωπινων Υπηρεσιών των Η.Π.Α.(U.S. Department of Health and Human Services) (Bethesda, Md., USA) αριθμός έκδοσης (CDC) 88—8395, ή οποιοδήποτε άλλο κατά τόπους σχετικό Εθνικό κανονισμό.
- Μεταχειριστείτε όλα τα δείγματα ως μολυσμένα.
- Αποφύγετε επαφή με αντιδραστήρια που περιέχουν υπεροξειδίου του υδρογόνου ή υδροχλωρικό οξύ. Σε περίπτωση επαφής με τέτοιου είδους αντιδραστήρια, πλυθείτε σχολαστικά με άφθονο νερό.
- Ακολουθείστε τις κατά τόπου οδηγίες για απομάκρυνση άχρηστου υλικού.

VARNINGAR OCH SÄKERHETSÅTGÄRDER

SE

Endast för *in vitro* diagnostik

- Endast för professionellt bruk
- Följ "U.S. Department of Health and Human Services (Bethesda, Md., USA) publikation (CDC) 88–8395" eller annan lokal eller nationell bestämmelse beträffande laboratoriesäkerhet.
- Hantera alla patientprover som potentiellt smittsamma.
- Vissa reagens innehåller natriumazid (NaN₃) som konserveringsmedel. Natriumazid kan reagera med bly- och kopparledningar och bilda explosiva metall-azider. Använd rikligt med vatten vid nedspolning i avloppet för att förhindra metall-azid bildning.
- Följ lokala bestämmelser för bortskaftande av avfall.

CanAg S100 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

SUMMARY AND EXPLANATION OF THE ASSAY

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 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 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).

Determination of S100B in serum has been shown to be clinically useful for prognosis and treatment monitoring of patients diagnosed with malignant melanoma (6-9). Studies also suggest that S100B may be useful in the management of patients with brain damage from eg. traumatic head injury, perinatal asphyxia, cardiac arrest, cardiac surgery and stroke (10-13).

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 patient samples are incubated together with biotinylated Anti-S100B monoclonal antibody (MAb) S23 in Streptavidin coated microtiter strips. S100B present in calibrators or samples is adsorbed to the Streptavidin coated microtiter wells 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 microtiter 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 S100B concentrations of patient 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

Streptavidin Microtiter Plate 1 Plate

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

12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

S100 Calibrators

6 vials, lyophilized

4 weeks at 2–8°C
3 months at –30°C or below

CAL	S100	A
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1 x 1 mL

CAL	S100	B
-----	------	---

1 x 1 mL

CAL	S100	C
-----	------	---

1 x 1 mL

CAL	S100	D
-----	------	---

1 x 1 mL

Component	Quantity	Storage and stability after first opening
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CAL	S100	E	1 x 1 mL
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CAL	S100	F	1 x 1 mL
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The lyophilised calibrators contain bovine S100B in a protein matrix with 0.02% NaN_3 as 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.

BIOTIN	Anti-S100
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Biotin Anti-S100	1 x 15 mL	2–8°C until expiry date stated on the vial
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Biotin Anti-S100 monoclonal antibody from mouse, approximately 2 µg/mL. Contains phosphate buffered saline (pH 7.2) with CaCl_2 , 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-S100
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Tracer, HRP Anti-S100	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock solution of HRP Anti-S100 monoclonal antibody from mouse, approximately 20 µg/mL. Contains preservatives. To be diluted with Tracer Diluent before use.

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.

Component	Quantity	Storage and stability after first opening
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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.

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- Handle all patient specimens as potentially infectious.
- Reagents contain sodium azide (NaN_3) 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 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. Microtiter plate shaker

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

2. Microtiter plate wash device

Automatic plate wash capable of performing 1, 3 and 6 washing cycles, or a semi manual microtiter plate 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 microtiter platewash is not used.

3. Microtiter plate 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 patient 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 respenser 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
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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

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

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 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 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 microtiter plate 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 patient 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 (± 10 min) at room temperature (20–25°C) with constant shaking of the plate using a microtiter plate 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 (± 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 (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
12. Immediately read the absorbance at 620 nm in a microtiter plate spectrophotometer.

Option

If the laboratory does not have access to a microtiter plate 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 microtiter plate 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

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 S100B as controls.

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 microtiter plate 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 patient specimen.

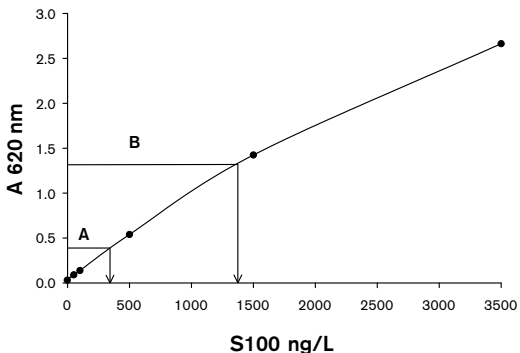
If samples in an initial analysis give S100 levels higher than Calibrator F (circa 3500 ng /L) the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate S100 concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the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

The level of S100 cannot be used as absolute evidence for the presence or absence of malignant disease, and the S100 test should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the management of patients, and the S100 test should not replace any established clinical examination.

Increases in serum S100B should be interpreted with caution for patients subject to trauma, such as bone fractures, burns, internal soft-tissue damage and surgery since these conditions are connected to significant release of S100B (14).

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

EXPECTED VALUES

S100B was measured in 94 healthy blood donors. The lower and upper extremes of the normal range were examined using IFCC recommended non-parametric statistical treatment. The reference interval contains the central 95% fraction of the reference distribution. Reference limits may accordingly be estimated as the 2.5% (lower) and 97.5% (upper) fractiles. These limits cut off a fraction of 2.5% of the values in each tail of the reference distribution. Non-parametric estimates:

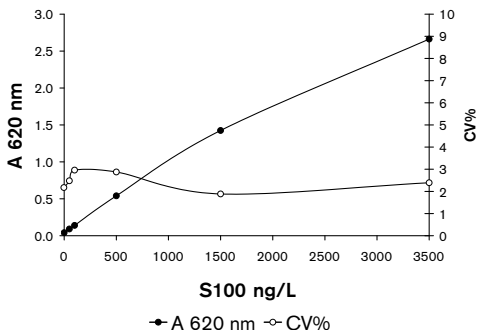
Fraction	Reference limit (ng/L)
2.5 th (lower)	25
97.5 th (upper)	91

It is recommended that each laboratory establish their own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc.

PERFORMANCE CHARACTERISTICS

Dose-response and precision profile

A typical calibration curve and precision profile obtained with the CanAg S100 EIA kit are shown below. The precision profile is based on random pipetting of calibrators into one microtiter plate, n=8.



Precision

Total precision was calculated according to NCCLS guideline EP5-A (15) using four levels of frozen pooled human serum containing added S100 and 22 different CanAg S100 EIA reagent combinations. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days.

Sample	Replicates	Mean (ng/L)	Within-run SD (ng/L)	Within-run CV %	Between-day SD (ng/L)	Between-day CV %
S100 1	80	70	2	2.5	2	2.2
S100 2	80	302	5	1.6	8	2.5
S100 3	80	1440	20	1.4	21	1.5
S100 4	80	2260	30	1.3	85	2.0

Detection limit

The detection limit of the CanAg S100 EIA is ≤ 10 ng/L defined as the concentration corresponding to the mean of the absorbance values of the S100 calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times \text{SD CAL A}}{\text{OD CAL B} - \text{OD CAL A}} \times [\text{CAL B}] \text{ ng/L}$$

Recovery

Spiked serum samples were prepared by adding human S100 antigen to normal serum samples. The recovery of the added antigen was in the range 97–105 %.

NOTE: recovery studies should **not** be performed using the kit calibrators.

Hook effect

No hook effect has been noticed with samples up to 150 000 ng/L at 620 nm or 7 000 000 ng/L at 405 nm.

NOTE: In very high samples the colour of the substrate will change from blue to greenish (and eventually yellow in extremely high samples). This will lead to a falsely low absorbance at 620 nm, and in extreme cases the absorbance may fall within the calibration curve range and noticed as a hook.

Linearity

Patient samples were serially diluted with normal human serum and analysed. The obtained values were within $\pm 10\%$ of the expected values.

Specificity

The CanAg S100 EIA is based on two mouse monoclonal antibodies specific for two different epitopes expressed in S100B, the catching MAb S23 and the detecting MAb S53. Thus the assay determines both S100A1B and S100BB without cross-reactivity with other forms of S100. The NCCLS guideline EP7-P (16) was followed to determine possible sources of interference. The following substances and concentrations were tested and found not to interfere with the test.

	Concentration with no significant ($\pm 10\%$) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	3.9 mg/mL

Method comparison

The CanAg S100 EIA was compared to the Sangtec 100. Ninety-eight human serum samples from patients with malignant melanoma, ranging in values from 0-8000 ng/L were measured and linear regression analyses of the results yielded:

$$\text{CanAg S100} = 0.4 \times \text{Sangtec 100} + 0.03 \quad r = 0.99$$

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by CanAg Diagnostics may affect the results, in which event CanAg 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.
6. Banfalavi T. et al., (2003) Use of serum S-100B protein levels to monitor the clinical course of malignant melanoma. *Eur J Cancer* 39:164-169.
7. Djureen-Mårtensson E., et al., (2001) Serum S-100b protein as a prognostic marker in malignant cutaneous melanoma. *J Clin Oncol* 19:824-831.
8. Hauschild A. et al., (1999) S100B protein detection in serum is significant prognostic factor in metastatic melanoma. *Oncology* 56:338-344.
9. Wunderlich MT., et al., (1999) Early Neurobehavioral Outcome after stroke is related to release of neurobiochemical markers of brain damage. *Stroke* 30:1190-1195.
10. Martens P et al., (1998) Serum S100 and neuron specific enolase for prediction of regaining consciousness after global cerebral ischemia. *Stroke* 29:2363-2366.
11. Rosén H. et al., (1998) Increased serum levels of the S-100 protein are associated with hypoxic brain damage after cardiac arrest. *Stroke* 29: 473-477.
12. Ingebrigtsen T. et al., (2000) The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. *Brain Inj* 14:1047-1055
13. Michetti F and Gazzolo D. (2002) S100B protein in biological fluids: A tool for perinatal medicine *Clin Chem* 48:2097-2104
14. Anderson R. et al., (2001) High serum S100B levels for trauma patients without head injuries *Neurosurgery* 48:1255-1258
15. National Committee for Clinical Laboratory Standards, Evaluation of Precision Performance of Clinical Chemistry Devices. Approved Guideline EP5-A (1999).
16. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation protocol Number 7, Vol. 6, No 13, August (1986).

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