



CanAg S100 EIA

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

REF **708-85**

Instructions for use. 2022-06

Read highlighted changes

EN	EXPLANATION OF SYMBOLS
BG	ОБЯСНЕНИЕ НА СИМВОЛИТЕ
CS	VÝZNAM SYMBOLŮ
DA	SYMBOLFORKLARING
DE	ERKLÄRUNG DER SYMBOLE
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	SIGNIFICADO DE LOS SÍMBOLOS
ET	SÜMBOLITE SELGITUS
FR	EXPLICATION DES SYMBOLES
HR	OBJAŠNJENJE SIMBOLA
HU	JELMAGYARÁZAT
IT	SPIEGAZIONE DEI SIMBOLI
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	SIMBOLU SKAIDROJUMS
NL	VERKLARING DER SYMBOLEN
NO	SYMBOLFORKLARING
PL	OBJAŚNIENIE SYMBOLI
PT	EXPLICAÇÃO DOS SÍMBOLOS
RO	SEMNIȚAȚIA SIMBOLURILOR
RU	ОБОЗНАЧЕНИЯ
SV	SYMBOLFÖRKLARING
SK	VÝZNAM SYMBOLOV
SL	RAZLAGA SIMBOLOV
SR	OBJAŠNJENJE SIMBOLA
TR	SEMBOLLERİN AÇIKLAMALARI



Use By/Годно до/Použitelné do/
Holdbar til/Verwendbar bis/
Ημερομηνία λήξης/Fecha
de caducidad/Kölblik kuni/
Utiliser jusque/Rok valjanosti/
Felhasznáható/Utilizzare entro/
Sunautoti iki/Izlietot līdz/Houdbaar
tot/Brukes innen/Użyć przed/
Prazo de validade/Expirã la/
Использовать до/Använd före/
Použite/né do/ Uporabno do/
Upotrebljivo do/Son Kullanna Tarihi

LOT

Batch code/Номер на партида/
Číslo šarže/Lotnummer/
Chargenbezeichnung/Αριθμός
Παρτίδας/Código de lote/Partii
kood/Code du lot/Kod serije/
Sarzszzám/Codice del lotto/
Partijos kodas/Partijas kods/Lot
nummer/Partikode/Kod partii/
Código do lote/Număr de lot/
Номер лота/Lotnummer/Číslo
šarže/Številka serije/Kod partije/
Parti Kodu



Date of manufacture/Дата на производство/Datum výroby/
Produktionsdato/Herstellungsdatum/
Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/
Date de fabrication/Datum proizvodnje/
Gyártási idő/Data di produzione/
Pagaminimo data/Ražošanas datums/
Productiedatum/Fremstillingsdato/
Data produkcji/Data de fabrico/Data fabricației/Дата производства/
Tilberkningsdatum/Datum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

REF

Catalogue number/Каталожен номер/
Katalógové číslo/Katalognummer/
Bestellnummer/Αριθμός καταλόγου/
Número de catálogo/Katalogi number/
Numéro de catalogue/Kataloški broj/
Katalógusszám/Numero di catalogo/
Katalogo numeris/Numurs katalogā/
Catalogusnummer/Katalognummer/
Numer katalogowy/Número do catálogo/
Număr de catalog/Номер по каталогу/
Produktnummer/Katalógové číslo/
Kataloška številka/Kataloški broj/
Katalog numarası



Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Inneholder tilstrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιχόμενα επαρκώς για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour «96» tests/Sadržaj dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per «96» saggi/Turinys skitas atlikti <96> tyrimus/Saturs pietiekams <96> testiem/Inhoud voldoende voor «96» testen/till «96» test/Tilstrækkelig innhold for <96> prøver/Wystarczy na wykonanie <96> testów/Conteúdo suficiente para «96» ensaios/Conținut suficient pentru 96 de teste/Содержит достаточные количества для «96» определений/Innehåller tillräckligt till «96» antal tester/Obsah postačuje na tento počet testov. <96>/Vsebina zadostuje za <96> testov/Sadržina dovoljna za <96> testova.<96> testleri için yeterli/içerir

Consult Instructions for Use/
Прочетете инструкцията за употреба/Konzultujte s návodem k použití/Se brugsanvisning/Siehe Gebrauchsanweisung/Συμβουλευτείτε τις Οδηγίες σχετικά με τη χρήση/
Consulte las instrucciones de uso/
Vt kasutusjuhendit/Consulte le mode d'emploi/Pročítajte upute za uporabu/Olvassa el a használati utasítást/
Consultare le istruzioni per l'uso/Dël naudojimo žiūrēkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene fer bruk/Sprawdź w instrukcji użycia/Consulte as Instruções de Utilização/Consultați instrucțiunile de utilizare/
Обратитесь к инструкции по применению/Se bruksanvisning/
Prečítajte si návod na používanie/
Pročitajte uputstvo za uporabu/
Kullanım Talimatlarına Bakınız



Temperature limitation/
Температурни граници/
Teplotní omezení/
Temperaturbegrænsning/
Temperaturbegrenzung/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Temperatuuri piirang/
Limite de température/
Temperaturno ograničenje/
Hőmérsékletre vonatkozó korlátozás/
Limiti di temperatura/
Temperatūriai apribojimai/
Temperatūras ierobežojums/
Temperaturbepërking/
Temperaturbegrensninger/
Temperatury graniczne/
Limite de temperatura/
Limite de temperatură/
Температурный режим/
Temperaturbegrænsning/
Teplotné obmedzenie
Omejitve temperature/
Temperaturno ograničenje/
Sıcaklık sınırlaması/

CONT

Contents of kit/Съдържание на набора/Obsah soupravy/Kittens indhold/Inhalt des Kits/Περιχόμενα του κιτ/Contenido del kit/Komplekt sisaladab/Contenu du kit/Sadržaj opreme/A készlet tartalma/Contenido del kit/Rinkinio turinys/
Komplekta saturs/Inhoud van de set/Settets innhold/Zawartość zestawu/Conteúdo do kit/Conținutul setului/
Компоненты набора/Kit innehåll/
Obsah súpravy/Vsebina kompleta/Sadržaj opreme/Kitin içindekiler



Biological risks/Биологическа опасност/Biologická rizika/Biologisk fare/Biologische Gefahren/Biologischो किवंनो/Riesgos biológicos/Biologilised ohud/Risques biologiques/Biolóikii rizic/Biológiai kockázatok/Rischio biológico/Biologinis pavojus/Biologiskais risks/Biologische risico's/Biologische risikoer/Zagrozenie biologiczne/Riscos biológicos/ Biologisk risk/Pericole biologice/Биологическая опасность/Biologicky rizikové/Biologické riziká/Biolóikii rizic/Biyojik riskler

ORIG HUM

Human/С човешки производ/Lidské/ Humant/Human/δύγυατα ανθρωπάς/
Humano/Inimpãritolu/Humaine/Ljudskog porijekla/Humán/Origine Umana/
Žmogaus kilmės/Cilvēku izcelsmes/
Human/Menneske/Ludzka/Humano/
Origine umană/Человеческого происхождения/Human/Ludské/
Humanega izvora/Ljudskog porekla/İnsan

ORIG MOU

From mouse/С миши производ/Mišji/
Fra mus/Maus/από ποντίκι/de raton/Hiirtelt/De souris/Mišjeg porjekla/
Egérböli/Murino/Pelės kilmės/No peles/
Van muizen/Fra mus/Mysia/Do rato/De la șoareci/Мышиного происхождения/
Från mus/Mýsie/Mišjega izvora/Mišjeg porekla/Faređen

ORIG BOV

Bovine/С говежди производ/Hovéz/!Bovin/Rind/από βοοειδή/Bovino/Veistelt/Bovine/Rogate stoke/Szarvasmarha/Bovino/Jaučio/No liellopa/Bovien/Bovin/Wolowy/Bovino/Origine bovină/крупного рогатого скота/Från ko/Hovädzie/Govejega izvora/Rogate krupne stoke/Bovin



Reconstitute with/Разтваряне с/Rozfedte pomoci/Rekonstituieres med/Rekonstituieren mit/Ανασύσταση με/Reconstituir con/Lahjendamine/Reconstituer avec/Rekonstituiraite s/Feloldashoz/Ricostituire con/Atkurti, ištirindas su/Atšikaidit ar/Reconstituite met/Rekonstituieres med/Odtvorzyć za pomocą/Reconstituir com/A se reconstitui cu/Разтворить в/Rekonstituera med/Rozriedte pomocou/Rekonstituiraite z/si/Ponovno formiranje sa/Yeniden oluştulur



Manufacturer/Производител/Výrobce/Producent/Hersteller/Κατασκευαστής/
Fabricante/Tootja/Fabricant/Proizvođač/
Gyártó/Fabbricante/Gamintojas/
Ražotājs/Fabrikant/Produsent/
Produsent/Fabricante/Producător/
Производител/Тililverkare/ Vyrroba/
Izdevalcav/Proizvođač/Üretici

CanAg S100 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

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

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
MICROPLATE		
Microplate	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.		

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
CAL S100 A	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	
CAL S100 E	1 x 1 mL	
CAL S100 F	1 x 1 mL	

The lyophilised 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.

BIOTIN Anti-S100	Quantity	Storage and stability after first opening
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-S100	Quantity	Storage and stability after first opening
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
DIL CONJ		
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		
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	Quantity	Storage and stability after first opening
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.

WASHBUF 25X	Quantity	Storage and stability after first opening
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 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.

CLP (1272/2008) HAZARD CLASSIFICATION

Information about CLP (1272/2008) HAZARD CLASSIFICATION can be found at the end of this document.

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 700-1100/min.

2. Microplate wash device

Automatic plate wash capable of performing 1 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.

An 8-channel pipette with disposable plastic tips for delivery of 350 µL is recommended if an automatic microplate washer 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. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.
 - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. It's highly recommended to use *strip* process mode and *overflow* wash mode with a dispensing volume of 800 µL. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
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.

Protocol Sheet

CanAg S100 EIA REF 708-85

Mix the components directly before use. Use shaking conditions according to the Instructions.

Step	Bottle/Plate	Procedure																																							
1. Prepare S100 Calibrators	CAL S100 A, B, C, D, E, F	Add 1 mL of distilled water to each vial and mix gently. Allow to stand for at least 15 minutes. NOTE: The exact concentration of each calibrator is stated on the label. Reconstituted stability: 4 weeks at 2-8°C.																																							
	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionized water.																																							
	CONJ Anti-S100 DIL CONJ	Mix 50 µL of Tracer, HRP Anti-S100 with 1mL of Tracer Diluent per strip:																																							
		<table border="1"> <thead> <tr> <th>No. of Strips</th> <th>Tracer, HRP Anti-S100 (µL)</th> <th>Tracer Diluent (mL)</th> </tr> </thead> <tbody> <tr><td>1</td><td>50</td><td>1</td></tr> <tr><td>2</td><td>100</td><td>2</td></tr> <tr><td>3</td><td>150</td><td>3</td></tr> <tr><td>4</td><td>200</td><td>4</td></tr> <tr><td>5</td><td>250</td><td>5</td></tr> <tr><td>6</td><td>300</td><td>6</td></tr> <tr><td>7</td><td>350</td><td>7</td></tr> <tr><td>8</td><td>400</td><td>8</td></tr> <tr><td>9</td><td>450</td><td>9</td></tr> <tr><td>10</td><td>500</td><td>10</td></tr> <tr><td>11</td><td>550</td><td>11</td></tr> <tr><td>12</td><td>600</td><td>12</td></tr> </tbody> </table>	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
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																																							
2. Wash	MICROPLA	Wash each well once with Wash Solution. Use manual or automatic washer.																																							
3. Add calibrators and samples	CAL S100 A, B, C, D, E, F	50 µL in each well																																							
4. Add Biotin Anti-S100	BIOTIN Anti-S100	100 µL in each well																																							
5. Incubate	MICROPLA	2 hour shaking at room temperature																																							
6. Wash	MICROPLA	Wash each well three times with Wash Solution Use manual or automatic washer.																																							
7. Add Tracer working solution	TRACER WORKING SOLUTION	100 µL in each well																																							
8. Incubate	MICROPLA	1 hour shaking at room temperature																																							
9. Wash	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.																																							
10. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well																																							
11. Incubate	MICROPLA	30 min shaking at room temperature																																							
12. Read absorbance	MICROPLA	620 nm																																							
Alt.12 Add Stop Solution	STOP	100 µL in each well																																							
Alt.13 Incubate	MICROPLA	1 min shaking at room temperature																																							
Alt.14 Read absorbance	MICROPLA	Read at 405 nm within 5 min																																							

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
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 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 (± 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 (± 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 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 5 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.

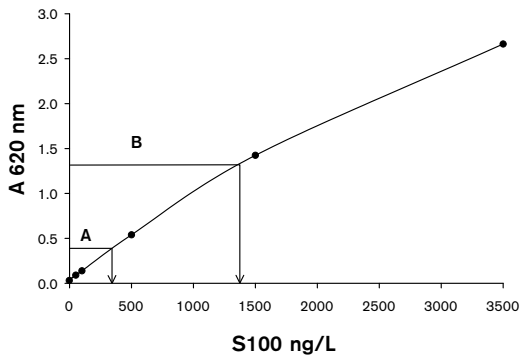
If samples in an initial analysis give S100 levels higher than Calibrator F (circa 3500 ng /L) the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate S100 concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the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

CLP (1272/2008) HAZARD CLASSIFICATION

The following warnings and precautions apply to

SUBS **TMB**

Hazard pictograms:



Signal word:

Danger

Hazard Statement:

Repr. 1B: H360D May damage the unborn child.

Prevention statement:

P202 Do not handle until all safety precautions have been read and understood.

Prevention:

P280 Wear protective gloves / protective clothing / eye protection / face protection.

Precautionary statement response:

P308+P313 IF exposed or concerned get medical advice/attention.

Precautionary statement disposal:

P501 Dispose of contents / container to an approved hazardous / special waste disposal facility in accordance with local and national regulations.

Restricted to professional users.

Hazardous substances: 2- Pyrrolidone

Other hazards

None of the mixtures in the kit contains any substances considered to meet the criteria classifying them as PBT and/or vPvB.

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.

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