FOR INFORMATION ONLY.
WHEN PERFORMING
THE ASSAY ALWAYS REFER
TO PACKAGE INSERT
SUPPLIED
WITH THE KIT

CanAg SCC EIA

REF 800-10

IVD

CE

Instructions for use. 2009-11

DE **Wenden Sie sich bitten an die deutsche Niederlassung um die geltende Gebrauchsanweisung zu erhalten.**

ES **Por favor contacte con su distribuidor para una versión válida de "Instrucciones de uso" en español**

IT **Contattare il proprio Distributore per ottenere la versione ufficiale della traduzione in lingua Italiana delle Istruzioni per l'Uso**

FR **Pour une version certifiée de la Notice en Français, veuillez contacter votre Distributeur.**

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 οδηγιων χρήσης**

SE **Vänligen kontakta Er distributör för gällande version av bruksanvisning på svenska.**

GB EXPLANATION OF SYMBOLS
DE BEDEUTUNG DER SYMBOLE
ES EXPLICACIÓN DE SÍMBOLOS
IT SIGNIFICATO DEI SIMBOLI
FR EXPLICATION DES SYMBOLES
NL PICTOGRAMMEN
DK SYMBOLFORKLARING
CS VYSVĚTLENÍ SYMBOLŮ
GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
PT INTERPRETAÇÃO DE SÍMBOLOS
HU JELMAGYARÁZAT
SE SYMBOLFÖRKLARING
PL INTERPRETACJA SYMBOLI
LT SIMBOLIŲ PAAIŠKINIMAI
RU ОБОНАЧЕНИЯ

Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Houdbaar tot/Holdbar til/
Použitelné do/Ημερομηνία λήξης/
Prazo de validade/Felhasználható
Bäst före datum/Użyć przed/
Sunaudoti iki/Использовать до

LOT

Batch code/
Chargenbezeichnung/
Codigo de lote/
Codice del lotto/Code du lot/
Lot nummer/Lotnummer/
Číslo šarže/Αριθμός Παρτίδας/
Código do lote/Sarzsszám
Lotnummer/Kod partii/Partijos
kodas/Номер лота

Date of manufacture/
Herstellungsdatum/
Fecha de fabricación/
Data di fabbricazione/
Date de fabrication/
Produktie datum/Produktionsdato/
Datum výroby/Ημερομηνία
Παραγωγής/Data de fabrico/
Gyártás időpontja/Tillverkningsdatum/
Data produkcji/Pagaminimo data/
Дата производства

REF

Catalogue number/Bestellnummer/
Número de catálogo/
Numero di catalogo/Référence du
catalogue/Catalogus nummer/Katalog-
nummer/Katalogové číslo/
Αριθμός καταλόγου/
Referência de catálogo/
Katalógusszám/Produktnummer/
Numer katalogowy/Katalogo numeris/
Номер по каталогу

Manufacturer/Hersteller/Fabricante/
Fabbricante/Fabricant/Fabrikant/
Producent/Výrobce/Κατασκευαστής/
Fabricante/Gyártó/Tillverkare/
Producent/Gamintojas/
Производитель

Contains sufficient for <96> tests/
Inhalt ausreichend für <96> Prüfungen/
Contenido suficiente para <96>
ensayos/Contenuto sufficiente per
“96” saggi/Contenu suffisant pour
“96” tests/Inhoud voldoende voor “96”
testen/Indeholder tilstrækkeligt
til ”96” test/Lze použít pro <96> testů/
Περιεχόμενο επαρκές για «96»
εξετάσεις/Conteúdo suficiente para
“96” ensaios/A doboz tartalma <96>
vizsgálat elvégzéséhez elegendő/
Innehåller tillräckligt till ”96” antal tester/
Wystarczy na wykonanie <96> testów/
Turinys skirtas atlikti <96> tyrimus
/Содержит достаточные количества
для «96» определений

IVD

In Vitro Diagnostic Medical Device/
In Vitro Diagnostikum/Producto sani-
tario para diagnóstico in vitro/
Dispositivo medico-diagnostico in vitro/
Dispositif médical de diagnostic in vitro/
Medisch hulpmiddel voor in-vitro
diagnostiek/Medicinsk udstyr til in
vitro-diagnostik/In Vitro diagnostický
zdravotnický prostředek /
In Vitro Διαγνωστικό Ιατροτεχνολογικό
προϊόν/Dispositivo médico para
diagnóstico in vitro/In vitro
diagnosztikum/Endast för in vitro-
diagnostik/Wyrób do diagnistyki In
Vitro/In Vitro Diagnostinė Medicinos
Priemonė/Только для диагностики
In Vitro

Temperature limitation/
Temperaturbegrenzung/
Límite de temperatura/
Limiti di temperatura/
Limites de température/
Temperatuurlimiet/
Temperaturbegrænsning/
Teplotní rozmezi od do/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Hőmérséklettartomány/
Temperaturbegränsning/
Przestrzegać zakresu temperatury/
Temperatūriniai apribojimai/
Температурный режим

Consult Instructions for Use/
Gebrauchsanweisung beachten/
Consulte las instrucciones de uso/
Consultare le istruzioni per l'uso/
Consulter les instructions d'utilisation/
Raadpleeg de gebruiksaanwijzing/
Se brugsanvisning/Viz návod k
použití/Συμβουλευτείτε τις οδηγίες
χρήσης/Consulte as instruções de
utilização/Nézze meg a Használati
utasítást/Se bruksanvisning/Sprawdź
w instrukcji obsługi/Dėl naudojimo
žiūrėkite instrukcijas/
Обратитесь к инструкции по
применению

Biological risks/Biogefährdung/
Riesgo biológico/Rischio biologico/
Risques biologiques/Biologisch
risico/Biologisk fare/
Biologicky nebezpečné
Βιολογικοί κίνδυνοι/Risco biológico
Biológiai kockázat/Biologisk risk/
Ryzyko biologiczne/Biologinis pavojus/
Биологическая опасность

CONT

Contents of kit/Inhalt/Contenido/
Contenudo/Contenu/Indhold/
ανιδραστήρια/Kit innehåll/
Rinkinio turinys/
Компоненты набора

ORIG | **MOU**

From mouse/der Maus/de ratón/
Murino/De souris/Mus/απο ποντίκι/
Från mus/Pelės kilmės/
Мышиного происхождения

ORIG | **HUM**

Human/Human/Humano/
Origine Umana/Humaine/Human
δείγματα αναφοράς/Human/
Žmogaus kilmės/
Человеческого происхождения

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.
- Follow local guidelines for disposal of all waste material.

WARNHINWEISE UND VORSICHTSMASSNAHMEN

DE

Für In-vitro-Diagnostik

- Nur für geschultes Fachpersonal.
- Bitte beachten Sie die Vorschriften zur Laborsicherheit in der Publikation Nr. (CDC) 88-8395 des US Department of Health and Human Services (Bethesda, MD, USA) oder andere gleichwertige regionale oder nationale Bestimmungen.
- Alle Patientenproben gelten als potenziell infektiös und sind entsprechend zu handhaben.
- Befolgen Sie die lokalen Richtlinien zur Entsorgung von anfallenden Abfallstoffen.

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.
- 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
- Seguire le normative vigenti relative all'eliminazione del materiale usato

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PRÉCAUTIONS D'EMPLOI ET MISE EN GARDE

FR

Pour un usage diagnostic 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 (Béthesda, 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.
- 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.
- Følg lokale regler for afskaffelse af alt affald.

ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ ΚΑΙ ΠΡΟΦΥΛΑΞΕΙΣ

GR

Για in vitro διαγνωσική χρήση

- Για επαγγελματική χρήση, μόνο.
- Παρακαλούμαι όπως επικαλεστείτε τις οδηγίες ασφαλούς λειτουγίας 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.
- Μεταχειριστήτε όλα τα δείγματα ως μολυσμένα.
- 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.
- Följ lokala bestämmelser för bortskaffande av avfall.

GB

CanAg SCC EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

IMPORTANT USER INFORMATION

SCC antigen is present in skin, sweat and saliva, and is easily distributed in aerosols (e.g. as a result of sneezing). In order to avoid false elevated values due to contamination, gloves should be used after opening the kit box and throughout the test procedure when handling reagent vials, microplate, pipette tips etc. In addition, all elevated values should be confirmed by repeat testing.

INTENDED USE

The CanAg SCC EIA kit is intended for the quantitative determination of squamous cell carcinoma (SCC) antigen in serum as an aid in the management of patients with squamous cell carcinoma.

SUMMARY AND EXPLANATION OF THE ASSAY

Squamous cell carcinoma antigen (SCC ag) is a group of glycoproteins with molecular weight ~45 kDa, belonging to the family of serine/cysteine -protease inhibitors (1). The protein was originally isolated by Kato and co-workers from human squamous cell carcinoma tissue and shown to consist of at least 10 subfractions differing in isoelectric point (2). More recent studies have shown that SCC antigen is composed of two distinct but highly homologous gene products, SCCA1 and SCCA2 with different inhibitor specificities (3).

SCC antigen is a serological marker of squamous cell carcinomas of the uterine cervix, vulva, lung, head & neck, and oesophagus (4-6). In squamous cell carcinoma of the uterine cervix, pre-treatment serum SCC ag may be used as an early stage prognostic factor (7) and the use of pre-treatment SCC ag have been suggested in order to select high-risk patients for adjuvant therapy (4). Further, for patients with elevated levels of SCC ag before start of treatment, the profile of SCC ag correlates with the response to radio- and chemo-therapy and measurement of SCC ag may thus be used to monitor the effect of therapy and for early detection of recurrent disease (4).

PRINCIPLE OF THE TEST

The CanAg SCC EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators and patient samples are incubated together with biotinylated Anti-SCC monoclonal antibody and horseradish peroxidase (HRP) labelled Anti-SCC monoclonal antibody in Streptavidin coated microstrips. 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 SCC 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 SCC concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg SCC 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 accordning 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		
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
SCC Calibrators	5 vials, lyophilized	4 weeks at 2–8°C 3 months at -20°C
CAL SCC A	1 x 0.75 mL	
CAL SCC B	1 x 0.75 mL	
CAL SCC C	1 x 0.75 mL	
CAL SCC D	1 x 0.75 mL	
CAL SCC E	1 x 0.75 mL	

The lyophilised calibrators contain human SCC in a Tris-HCl buffered salt solution containing bovine serum albumin, excipient, an inert yellow dye and 0.01% methyl-isothiazolone (MIT) as preservative. To be reconstituted with water before use. **NOTE:** The exact SCC concentration is lot specific and is indicated on the label of each vial.

BIOTIN Anti-SCC		
Biotin Anti-SCC	1 x 15 mL	2–8°C until expiry date stated on the vial

Biotin Anti-SCC monoclonal antibody from mouse, approximately 1 µg/mL. Contains phosphate buffered saline (pH 7.2), bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert blue dye and 0.01% methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-SCC before use.

CONJ Anti-SCC		
Tracer, HRP Anti-SCC	1 x 0.75 mL	2–8°C until expiry date stated on the vial

Stock solution of HRP Anti-SCC monoclonal antibody from mouse, approximately 40 µg/mL. Contains preservatives. To be mixed with Biotin Anti-SCC before use.

Component	Quantity	Storage and stability after first opening
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		
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		
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 in vitro diagnostic use.

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

SPECIMEN COLLECTION AND HANDLING

The CanAg SCC 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 1 day. For longer periods it is recommended to store the samples at –70°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 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.
   The Nunc Immuno-8 manual strip washer 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 SCC 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 SCC 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. 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 SCC EIA REF 800-10

Prepare the components directly before use. Use wash and incubation conditions according to the Instructions.

Step	Bottle/Plate	Procedure
1. Prepare SCC Calibrators	CAL | SCC A, B, C, D, E	Add 0.75 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. This value of the calibrators should be used for calculations.
2. Prepare Wash Solution	WASHBUF | 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.
3. Prepare Antibody Solution	CONJ | Anti-SCC BIOTIN | Anti-SCC	Mix 50 µL of Tracer, HRP Anti-SCC with 1 mL of Biotin Anti-SCC per strip:

No. of Strips	HRP Anti-SCC (µL)	Biotin Anti-SCC (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
4. Wash	MICROPLA	Wash each well once with Wash Solution
5. Add calibrators and samples	CAL | SCC A, B, C, D, E	25 µL in each well
6. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well
7. Incubate	MICROPLA	1 hour shaking at room temperature
8. Wash	MICROPLA	Wash each well six times with Wash Solution
9. Add TMB HRP-Substrate	SUBS | TMB	100 µL in each well
10. Incubate	MICROPLA	30 min shaking at room temperature
11. Read absorbance	MICROPLA	620 nm
Alt.11 Add Stop Solution	STOP	100 µL in each well
Alt.12 Incubate	MICROPLA	1 min shaking at room temperature
Alt.13 Read absorbance	MICROPLA	Read at 405 nm within 15 min

Preparation of reagents	Stability of prepared reagent
SCC Calibrators	4 weeks at 2–8°C 3 months at –20°C or below

Add exactly 0.75 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.

Antibody Solution	3 weeks at 2–8°C

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-SCC with 1 mL of Biotin Anti-SCC per strip (see table below and the Protocol Sheet):

No. of Strips	Tracer, HRP Anti-SCC (µL)	Biotin Anti-SCC (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 Antibody Solution.

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

NOTE: The Antibody Solution is stable for 3 weeks at 2–8°C. Do not prepare more Antibody 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 SCC Calibrators, Wash Solution and Antibody 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 25 µL of the SCC Calibrators (CAL A, B, C, D, E) 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					
B	Cal A	Cal E					
C	Cal B	Unk1					
D	Cal B	Unk1					
E	Cal C	Unk2					
F	Cal C	Unk2					
G	Cal D	Etc.					
H	Cal D						

4. Add 100 µL of Antibody Solution 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 1 hour (± 5 min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
6. Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.
7. Add 100 µL 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.
8. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
9. 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. 9. 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 SCC EIA measures concentrations between 0.3 and 50 µg/L. If SCC 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 SCC 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 SCC antigen, CanAg SCC 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 SCC Calibrators.
For automatic calculation of SCC 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 µg/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 µg/L.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/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 SCC calibrator against the corresponding SCC concentration (in µg/L), see figure below. The unknown SCC concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

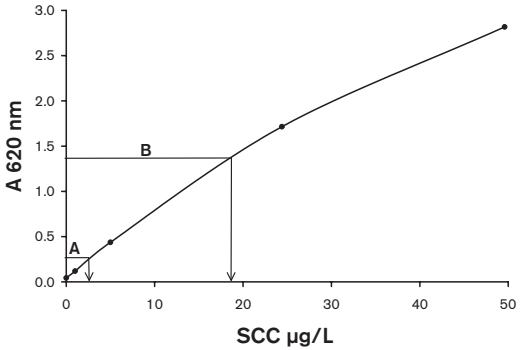
If samples in an initial analysis give SCC levels higher than 50 µg/L the samples should be diluted 1/10 with normal human serum and reanalyzed to obtain the accurate SCC concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the endogenous SCC concentration.

The SCC concentration of the undiluted sample is calculated as:

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

Example of results

Specimen	Calibrator values	Mean abs value (A)	SCC (µg/L)
CAL SCC A	0 µg/L	0.043	
CAL SCC B	1 µg/L	0.119	
CAL SCC C	5 µg/L	0.437	
CAL SCC D	24 µg/L	1.715	
CAL SCC E	50 µg/L	2.818	
Specimen A		0.245	2.6
Specimen B		1.363	18.3



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

LIMITATIONS OF THE PROCEDURE

SCC antigen is present in normal squamous cell epithelia and elevated SCC antigen may be found in skin disorders involving hyperkeratinization eg. psoriasis and eczema. Elevated levels also occur in benign conditions such as inflammatory lung disease and liver or renal insufficiency (4, 9).

The level of SCC thus cannot be used as absolute evidence for the presence or absence of malignant disease, and the SCC 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 SCC test should not replace any established clinical examination.

SCC antigen is present in skin, sweat and saliva, and is easily distributed in aerosols (e.g. as a result of sneezing). In order to avoid false elevated values due to contamination, gloves should be used throughout the test procedure when handling reagent vials, microplate, pipette tips etc. In addition, all elevated values should be confirmed by repeat testing.

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

CanAg SCC EIA was used to measure SCC antigen in 175 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. The upper reference limit was accordingly estimated as the 97.5% upper fractile.

	Mean (µg/L)	SD (µg/L)	Median (µg/L)	Range (µg/L)	Upper reference limit (µg/L)
Healthy blood donors n=175	0.58	0.24	0.54	0.16–1.5	1.2 µg/L

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. It should also be borne in mind that the individual patient's own baseline results provides the most important reference point for interpretation of marker results.

PERFORMANCE CHARACTERISTICS

Precision

Total precision was calculated according to NCCLS guideline EP5-A (10) using four levels of frozen pooled human serum containing added human SCC antigen and 18 different CanAg SCC EIA reagent combinations. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days.

Sample	Replicates	Mean (µg/L)	Within-run SD (µg/L)	Within-run CV %	Between-day SD (µg/L)	Between-day CV %
SCC 1	80	2.62	0.05	1.9	0.04	1.3
SCC 2	80	7.77	0.16	2.0	0.15	1.9
SCC 3	80	17.7	0.34	1.9	0.20	1.1
SCC 4	80	30.2	0.71	2.4	0.38	1.3

Detection limit

The detection limit of the CanAg SCC EIA is $\leq$ 0.3 µg/L defined as the concentration corresponding to the mean of the absorbance values of the SCC calibrator A plus 2 standard deviations according to formula:

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

Recovery

Spiked serum samples were prepared by adding human SCC antigen to normal serum samples. The recovery of the added antigen was in the range 90–110 %.

Hook effect

No hook effect has been noticed with samples up to 50 000 µg/L. **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 90–110% of the expected values.

Specificity

The CanAg SCC EIA is based on two mouse monoclonal antibodies, the catching MAb SCC 140 and the detecting MAb SCC 107 (11). The NCCLS guideline EP7-P (12) 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 (± 10%) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	5 mg/mL

Method comparison

The CanAg SCC EIA was compared to the Imx SCC MEIA.
For 72 human samples ranging in values from 0–4 µg/L, linear regression analyses of the results yielded:
CanAg SCC = 1.02 x Imx SCC + 0.03 r =0.86

For 138 human samples ranging in values from 0–50 µg/L, linear regression analyses of the results yielded:
CanAg SCC = 0.82 x Imx SCC + 0.06 r =0.98

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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