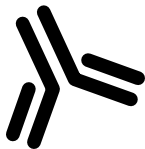


**FOR INFORMATION ONLY.
WHEN PERFORMING
THE ASSAY ALWAYS REFER
TO PACKAGE INSERT
SUPPLIED
WITH THE KIT**



CanAg SCC EIA

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

REF

800-85

Instructions for use. 2006-10

Enzyme immunometric assay kit
For 96 determinations

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



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

LOT

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



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

REF

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



Manufacturer/Hersteller/Fabricante/
Fabbriante/Fabricant/
Προδουcent/τασκευαστής/
Tilverkare



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



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



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



From mouse/der Maus/de ratón/
Murino/De souris/Mus/αΠΟ ΠΟΝΤΙΚΙ/
Från mus



Human/Human/Humano/
Origine Umana/Humaine/Human
δείγματα αναφοράς/Human

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.

INTENDED USE

The CanAg SCC EIA kit is intended for the quantitative determination of squamous cell carcinoma (SCC) antigen in serum.

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).

PRINCIPLE OF THE TEST

The CanAg SCC EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators and samples are incubated together with biotinylated Anti-SCC monoclonal antibody and horseradish peroxidase (HRP) labelled Anti-SCC monoclonal antibody in Streptavidin coated microstrips (4). 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 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 according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first opening
MICROPLA		
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			
<table border="1"><tr><td>CAL</td><td>SCC</td><td>A</td></tr></table>	CAL	SCC	A	1 x 0.75 mL	
CAL	SCC	A			
<table border="1"><tr><td>CAL</td><td>SCC</td><td>B</td></tr></table>	CAL	SCC	B	1 x 0.75 mL	
CAL	SCC	B			
<table border="1"><tr><td>CAL</td><td>SCC</td><td>C</td></tr></table>	CAL	SCC	C	1 x 0.75 mL	
CAL	SCC	C			
<table border="1"><tr><td>CAL</td><td>SCC</td><td>D</td></tr></table>	CAL	SCC	D	1 x 0.75 mL	
CAL	SCC	D			
<table border="1"><tr><td>CAL</td><td>SCC</td><td>E</td></tr></table>	CAL	SCC	E	1 x 0.75 mL	
CAL	SCC	E			

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
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Biotin Anti-SCC	1 x 15 mL	2–8°C until expiry date stated on the vial
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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
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Tracer, HRP Anti-SCC	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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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
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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.

WARNINGS AND PRECAUTIONS

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

- Please refer to the US Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all serum specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

SPECIMEN COLLECTION AND HANDLING

The CanAg 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, or a semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid.

The Nunc Immuno-8 manual strip washer is recommended if an automatic microplatewash is not used.

3. Microplate spectrophotometer

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

4. Precision pipettes

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

5. Distilled or deionized water

For reconstitution of 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 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 shake it carefully.

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.

Protocol Sheet

CanAg SCC EIA REF 800-85

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

Step	Bottle/Plate	Procedure																					
1. Prepare SCC Calibrators	<table border="1"><tr><td>CAL</td><td>SCC</td></tr></table> <p>A, B, C, D, E</p>	CAL	SCC	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.																			
CAL	SCC																						
2. Prepare Wash Solution	<table border="1"><tr><td>WASHBUF</td><td>25X</td></tr></table>	WASHBUF	25X	Dilute 50 mL of Wash Concentrate with 1 200 mL of distilled water or deionized water.																			
WASHBUF	25X																						
3. Prepare Antibody Solution	<table border="1"><tr><td>CONJ</td><td>Anti-SCC</td></tr><tr><td>BIOTIN</td><td>Anti-SCC</td></tr></table>	CONJ	Anti-SCC	BIOTIN	Anti-SCC	Mix 50 µL of Tracer, HRP Anti-SCC with 1 mL of Biotin Anti-SCC per strip:																	
CONJ	Anti-SCC																						
BIOTIN	Anti-SCC																						
	<table border="1"><thead><tr><th>No. of Strips</th><th>HRP Anti-SCC (µL)</th><th>Biotin Anti-SCC (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></tbody></table>	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	
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																					

0	0
7	350
8	400
9	450
10	500
11	550
12	600

4. Wash	MICROPLA	Wash each well once with Wash Solution
5. Add calibrators and samples	CAL SCC A, B, C, D, E	25 μ L in each well
6. Add Antibody Solution	ANTIBODY SOLUTION	100 μ L 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 μ L 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 μ L 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
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Pour the 50 mL Wash Concentrate into a clean container and dilute 25- fold by adding 1200 mL of distilled or deionized water to give a buffered Wash Solution.

Antibody Solution	3 weeks at 2–8°C
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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 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 unknown samples (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E					
B	Cal A	Cal E					
C	Cal B	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 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.
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 μ L of Stop Solution. Mix and read absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg SCC EIA measures concentrations between 0.3 and 50 μ g/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 specimen.

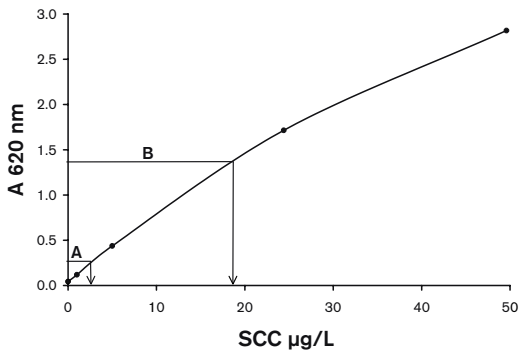
If samples in an initial analysis give SCC levels higher than 50 µg/L the samples should be diluted 1/10 with normal human serum and reanalyzed to obtain the accurate SCC concentration. **NOTE:** The sample used for dilution should also be measured in order to determine the endogenous SCC concentration.

The SCC concentration of the undiluted sample is calculated as:

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

Example of results

Specimen			Calibrator values	Mean abs value (A)	SCC ($\mu\text{g/L}$)
CAL	SCC	A	0 $\mu\text{g/L}$	0.043	
CAL	SCC	B	1 $\mu\text{g/L}$	0.119	
CAL	SCC	C	5 $\mu\text{g/L}$	0.437	
CAL	SCC	D	24 $\mu\text{g/L}$	1.715	
CAL	SCC	E	50 $\mu\text{g/L}$	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 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.

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

WARRANTY

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

LITERATURE REFERENCES

1. Suminami Y., Kishi F., Sekiguchi K., Kato H. (1991) Squamous cell carcinoma antigen is a new member of the serine protease inhibitors. *Biochem Biophys Res Commun* 181, 51-58.
2. Kato H. and Torigoe T. (1977) Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. *Cancer* 40, 1621-1628.
3. Schneider S.S., Schick C., Fish K.E., Miller E., Pena J.C., Treter S.D., Hui S.M., Silverman G.A. (1995) A serine protease inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. *Proc Natl Acad Sci USA*, 92, 3147-3151.
4. Røijer E., Nilsson K., Oskarsson M., Dahlén U., Andersson I., Nilsson O. (2003) Development of Monoclonal Antibodies and Immunoassays against different forms of Squamous Cell Carcinoma Antigens (SCCA). *Tumor Biol* 24, p. 83.



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