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

CanAg Free PSA EIA

REF **350-10**

IVD

CE 0197

Instructions for use. 2013-09

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	SEMNIFICAŢIA SIMBOLURILOR
RU	ОБОНАЧЕНИЯ
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/Kõlblik kuni/ Utiliser jusque/Rok valjanosti/ Felhasználható/Utilizare entro/ Sunaudoti iki/Izlietot līdz/Houdbaar tot/Brukes innen/Użyć przed/ Prazo de validade/Expiră la/ Использовать до/Använd före/ Použite´né do/ Uporabno do/ Upotrebljivo do/Son Kullanma Tarihi

LOT

Batch code/Номер на партида/ Číslo šarže/Lotnummer/ Chargenbezeichnung/Αριθμός Παρτίδας/Código de lote/Partii kood/Code du lot/Kod serije/ Sarzsszám/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 fabricaţiei/Дата производства/ Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

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

IVD

In Vitro Diagnostic Medical Device/ Медицински уред за диагностика ин витро/Diagnostický zdravotnický prostředek in vitro/Medicinsk udstyr til in vitro-diagnostik/In-vitro-Diagnostikum/ Ιατροτεχνολογικό προϊόν για διάγνωση In Vitro/Dispositivo médico para diagnóstico in vitro/In vitro diagnostiline meditsiiniseade/Dispositif médical de diagnostic in vitro/Diagnostički medicinski uređaj In Vitro/In vitro orvosdiagnosztikai eszköz/Dispositivo medico per test diagnostici in vitro/In Vitro Diagnostinė Medicinos Priemonė/Medicīniska ierīce in vitro diagnostikai/In vitro-diagnostisch medisch instrument/In vitro diagnostisk medisinsk utstyr/Wyrób medyczny do diagnostyki in vitro/Dispositivo Médico de Diagnóstico In Vitro/Dispozitiv medical pentru diagnostic in vitro/Только для диагностики In Vitro/Endast för in vitro-diagnostik/ Zdravotnícka pomôcka na diagnostiku in vitro/In vitro diagnostični pripomoček/Diagnostički medicinski uređaj In Vitro/<96> testleri için yeterlilik içerir

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilstrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιεχόμενο επαρκές για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour “96” tests/Sadrži dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per “96” saggi/Turinys skirtas atlikti <96> tyrimus/ Saturs pietiekams <96> testiem/Inhoud voldoende voor “96” testen/til “96” test/ Tilstrekkelig innhold for <96> prøver/ Wystarczy na wykonanie <96> testów/ Conteúdo suficiente para “96” ensaios/ Conţinut 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 yeterlilik içerir

REF

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

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/Consulter le mode d'emploi/Pročitajte upute za uporabu/ Olvassa el a használati utasítást/ Consultare le istruzioni per l'uso/Dėl naudojimo žiūrėkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene før bruk/Sprawdzić w instrukcji użycia/ Consulte as Instruções de Utilização/ Consultaţi instrucţiunile de utilizare/ Обратитесь к инструкции по применению/Se bruksanvisning/ Prečítajte si návod na používanie/ Pročitajte uputstvo za upotrebu/ Kullanım Talimatlarına Bakınız

CONT

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

Biological risks/Биологическа опасност/Biologická rizika/Biologisk fare/Biologische Gefahren/Βιολογικοί κίνδυνοι/Riesgos biológicos/ Bioloogilised ohud/Risques biologiques/ Biološkli rizici/Biológiai kockázatok/Rischi biologici/Biologinis pavojus/Bioloģiskais risks/Biologische risico's/Biologiske risikoer/Zagrożenie biologiczne/Riscos biológicos/ Biologisk risk/Pericole biologice/Биологическая опасность/ Biologicky rizikové/Biologické riziká/ Biološkli rizici/Biyolojik riskler

ORIG | HUM

Human/С човешки произход/Lidské/ Humant/Human/δείγματα αναφοράς/ Humano/Inimpäritolu/Humaine/Ljudskog porjekla/Humán/Origine Umana/ Žmogaus kilmės/Cilvēku izcelsmes/ Human/Menneske/Ludzka/Humano/ Origine umană/Человеческого происхождения/Human/Ľudské/ Humanega izvora/Ljudskog porekla/İnsan

ORIG | MOU

From mouse/С миши произход/Myší/ Fra mus/Maus/απο ποντίκι/de ratón/ Hiirtelt/De souris/Mišijeg porjekla/ Egérből/Murino/Pelės kilmės/No peles/ Van muizen/Fra mus/Mysia/Do rato/De la şoareci/Мышиного происхождения/ Från mus/Myšie/Mišjega izvora/Mišijeg porekla/Fareden

ORIG | BOV

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

Reconstitute with/Разтваряне с/ Rozřeďte pomocí/Rekonstitueres med/ Rekonstituieren mit/Ανασύσταση με/ Reconstituir con/Lahjendamine/ Reconstituer avec/Rekonstituirajte s/ Feloldáshoz/Ricostituire con/Atkurti, ištirpdant su/Atšķaidīt ar/Reconstitutie met/Rekonstitueres med/Odtworzyć za pomocą/Reconstituir com/A se reconstitui cu/Растворить в/ Rekonstituera med/Rozrieďte pomocou/ Rekonstituirajte z/s/Ponovno formiranje sa/Yeniden oluşturulur

Manufacturer/Производител/Výrobce/ Producent/Hersteller/Κατασκευαστής/ Fabricante/Tootja/Fabricant/Proizvođač/ Gyártó/Fabbricante/Gamintojas/ Ražotājs/Fabrikant/Produsent/ Producent/Fabricante/Producător/ Производитель/Tillverkare/ Výrobca/ Izdelovalec/Proizvođač/Üretici

EN

CanAg Free PSA EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg Free PSA EIA kit is intended for the quantitative determination of Free PSA (Prostate Specific Antigen) in human serum.

SUMMARY AND EXPLANATION OF THE ASSAY

PSA is a 32 kDa single chain glycoprotein serine protease with a chymotrypsin like specificity produced by the secretory epithelium of the prostate gland (1). PSA is normally secreted into the seminal fluid and plays a functional role in the cleavage of the seminal vesicle proteins and the liquefaction of the seminal coagulum (2). Only low levels of PSA are normally present in the blood stream, and increasing serum concentrations indicate prostatic pathology, including benign prostatic hyperplasia and cancer of the prostate. Determination of PSA is now widely used for detection and management of patients with prostatic cancer and considered as the superior serological marker for cancer of the prostate (3).

PSA has been shown to form stable complexes with different antiproteases and the dominating portion of PSA in patient serum occurs in complex with α_1-antichymotrypsin (PSA-ACT) (4). However there are large variations in the relation between Free PSA and PSA-ACT complex between different individuals. A number of studies have found that the proportion of Free PSA is higher in benign prostatic disease as compared to prostatic cancer (4, 5). The CanAg Free PSA EIA is an assay for specific determination of Free PSA without cross reactivity with PSA-ACT complex (6).

PRINCIPLE OF THE TEST

The CanAg Free PSA EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, controls and patient samples are incubated together with biotinylated Anti-Free PSA monoclonal antibody and horseradish peroxidase (HRP) labeled Anti-PSA 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 Free PSA present in the samples.

The colour intensity is determined in a microplate spectrophotometer at 450 nm.

Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The Free PSA concentration of patients samples are then read from the calibration curve.

REAGENTS

- Each CanAg Free PSA 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.

Free PSA Calibrators			6 vials	2–8° C until expiry date stated on the vials
CAL	PSA	0	0 µg/L 1 x 0.75 mL	
CAL	PSA	0.3	0.3 µg/L 1 x 0.75 mL	
CAL	PSA	1	1 µg/L 1 x 0.75 mL	
CAL	PSA	2	2 µg/L 1 x 0.75 mL	
CAL	PSA	5	5 µg/L 1 x 0.75 mL	
CAL	PSA	10	10 µg/L 1 x 0.75 mL	

Human Free PSA in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
Free PSA Controls	2 vials	2–8° C until expiry date stated on the vials
CONTROL FPSA 1	1 x 0.75 mL	
CONTROL FPSA 2	1 x 0.75 mL	

Human Free PSA in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

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

Biotin Anti-Free PSA monoclonal antibody from mouse, approximately 1.5 µg/mL. Contains Phosphate buffered saline (pH 7.2), 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-FPSA		
Tracer, HRP Anti-Free PSA	1 x 0.75 mL	2–8° C until expiry date stated on the vial

Stock solution of HRP Anti-PSA monoclonal antibody from mouse, approximately 20 µg/mL. Contains preservatives. To be mixed with Biotin Anti-Free PSA prior to 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.

Component	Quantity	Storage and stability after first opening
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.

Caution

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

SPECIMEN COLLECTION AND HANDLING

CanAg Free PSA 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 and at -20° C for 12 months. For longer periods store samples at -70° C or below. Samples should not be stored in a self-defrosting freezer. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

Elevated levels of Free PSA may be expected following manipulation of the prostate. It is therefore recommended that blood be drawn before digital rectal examination. Following surgical manipulation of the prostate, such as needle biopsy or transurethral resection it is recommended to wait ≥ than 6 weeks before drawing blood for Free PSA testing (7). It should be taken into account that Finasteride treatment of BPH have been shown to decrease Free PSA levels (7).

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous, approximately 700-1100 oscillations/min .
2. **Microplate wash device**
   Automatic plate washer 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 450 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 preparation of Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg Free PSA 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, controls 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 Free PSA EIA REF 350-10

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

Step	Vial/Plate	Procedure
1. Prepare wash solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.
Prepare Antibody solution	CONJ Anti-FPSA BIOTIN Anti-FPSA	Mix 50 µL of Tracer, HRP Anti-Free PSA, with 1 mL of Biotin Anti-Free PSA per strip:

No. of Strips	Tracer, HRP Anti-Free PSA (µL)	Biotin Anti-Free PSA (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
3.	Add calibrators, controls and samples	CAL | PSA 0, 0.3, 1, 2, 5, 10 CONTROL | FPSA 1, 2	50 µL in each well
4.	Add Antibody solution	ANTIBODY SOLUTION	100 µL in each well
5.	Incubate	MICROPLA	1 hour shaking at room temperature
6.	Wash	MICROPLA	Wash each well six times with wash solution
7.	Add TMB HRP-Substrate	SUBS | TMB	100 µL in each well
8.	Incubate	MICROPLA	30 min shaking at room temperature
9.	Add Stop Solution	STOP	100 µL in each well
10.	Incubate	MICROPLA	1 min shaking at room temperature
11.	Read absorbance	MICROPLA	Read at 450 nm within 15 min.

Preparation of reagents	Stability of prepared reagent
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 Free PSA with 1 mL of Biotin Anti-Free PSA per strip (see table below and the Protocol Sheet).

No. of Strips	Tracer, HRP Anti-Free PSA (µL)	Biotin Anti-Free PSA (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-Free PSA into the vial of Biotin Anti-Free PSA and mix gently. Make sure that all of the Tracer is transferred to the vial of Biotin Anti-Free PSA.

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, controls 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 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 50 µL of the Free PSA Calibrators (CAL 0, 0.3, 1, 2, 5, 10), controls (c) and patient samples (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	CAL 0	CAL 5	Unk1				
B	CAL 0	CAL 5	Unk1				
C	CAL 0.3	CAL 10	Unk2				
D	CAL 0.3	CAL 10	Unk2				
E	CAL 1	C1	Etc.				
F	CAL 1	C1					
G	CAL 2	C2					
H	CAL 2	C2					

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 (± 10 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. Add 100 µL of Stop Solution. Mix and read the absorbance at 450 nm in a microplate spectrophotometer within 15 minutes after addition of Stop Solution.

Measurement range

The CanAg Free PSA EIA measures concentrations between 0.03 and 10 µg/L. If Free PSA concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human male serum prior to analysis. **NOTE:** The serum used for dilution should also be measured in order to determine the endogenous Free PSA concentration (see "Calculation of results").

Quality Control

Free PSA Control 1 and 2 may be used for validation of the assay series. Ranges of expected results are indicated on the vial labels. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated. Each laboratory may in addition prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

Reference material

The 1st International Standard 96/668 may be used as a reference standard. Values for Free PSA Calibrators and Controls were assigned against a set of in-house reference standards whose values are traceable to the 1st International Standard.

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 Free PSA Calibrators.
For automatic calculation of Free PSA results it is recommended to use either of the following methods:

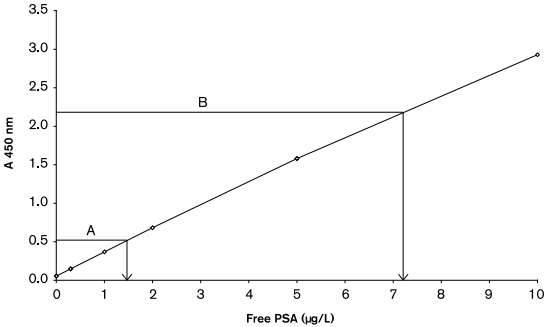
- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Quadratic 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 Free PSA calibrator against the corresponding Free PSA concentration (in µg/L), see figure below. The unknown Free PSA concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

Example of results

Specimen	Calibrator values	Mean abs value (A)	Free PSA (µg/L)
CAL PSA 0	0 µg/L	0.054	
CAL PSA 0.3	0.3 µg/L	0.148	
CAL PSA 1	1 µg/L	0.369	
CAL PSA 2	2 µg/L	0.683	
CAL PSA 5	5 µg/L	1.580	
CAL PSA 10	10 µg/L	2.930	
Specimen A		0.522	1.480
Specimen B		2.181	7.147



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

Calculation of results with diluted samples

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

The Free PSA concentration of the undiluted sample is calculated as:

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

LIMITATIONS OF THE PROCEDURE

The level of Free PSA alone should not be used as evidence for the presence or absence of malignant disease. 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. The Free PSA test should not replace any established clinical examination.

The calibrators of the CanAg Free PSA EIA kit should not be used for recovery studies of Free PSA. For recovery studies it is recommended to use a highly elevated patient sample.

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

Free PSA measurements may be used in conjunction with an equimolar test such as CanAg PSA EIA (340-10) for total PSA in order to generate the ratio of Free PSA/Total PSA. Serum specimens from 52 men objectively diagnosed with benign prostate hyperplasia (BPH) and 77 men diagnosed with prostate cancer (PCa) were analysed using CanAg PSA EIA and CanAg Free PSA EIA:

Diagnosis (n)	FPSA/TPSA			FPSA/TPSA	
	Median	Min.	Max.	Mean	(95 % confidence interval)
BPH (52)	0.18	0.04	0.42	0.19	(0.17–0.21)
PCa (77)	0.09	0.02	0.53	0.12	(0.10–0.14)

The choice of a cut-off to be used in clinical practice depends upon the clinical application, i.e. whether optimised sensitivity or specificity is desired. Sensitivities (% PCa correctly detected) and Specificities (% BPH correctly detected) for different FPSA/TPSA ratio cut-offs are shown below:

FPSA/TPSA cut-off	Clinical specificity (BPH > cut-off) n	%	(95 % confidence interval)	Clinical sensitivity (PCa ≤ cut-off) n	%	(95 % confidence interval)
0.23	14 (52)	27	(16–41)	69 (77)	90	(81–95)
0.16	36 (52)	69	(55–81)	64 (77)	83	(73–91)
0.08	48 (52)	92	(81–98)	30 (77)	39	(28–51)

It is recommended that each laboratory investigate the transferability of the above expected values to its own patient population and assay performance (7).

PERFORMANCE CHARACTERISTICS

Precision

Total precision was calculated according to NCCLS guideline EP5-A (8) using four levels of frozen pooled human serum containing added Free PSA and six different CanAg Free PSA 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 %
Free PSA 1	80	0.38	0.01	1.9	0.01	3.0
Free PSA 2	80	1.44	0.02	1.6	0.04	2.6
Free PSA 3	80	3.46	0.05	1.6	0.08	2.3
Free PSA 4	80	6.91	0.09	1.3	0.12	1.8

Detection limit

The detection limit of the CanAg Free PSA EIA is < 0.03 µg/L defined as the concentration corresponding to the mean of the absorbance values of the Free PSA calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 0.3 - OD\ CAL\ 0} \times 0.3\ \mu g/L$$

Recovery

Spiked serum samples were prepared by adding aliquots of samples with highly elevated Free PSA to normal male serum samples. The recovery of the antigen was within ± 15 % of the expected values. **Note:** Recovery studies should **not** be performed using the kit calibrators.

Hook effect

No hook effect has been noticed with samples up to > 5000 µg/L.

Linearity

Patient samples were diluted with normal male human serum and analysed. The obtained values were within ± 10 % of the expected values.

Specificity

The CanAg Free PSA EIA is based on two mouse monoclonal antibodies, PSA30 and PSA66, directed against two distinct epitopes exposed in Free PSA. This antibody combination provides an assay specific for Free PSA showing <1 % cross-reactivity to the PSA-ACT complex (6). The NCCLS guideline EP7-P (9) 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	0.4 mg/mL
Hemoglobin	5 mg/mL

Method comparison

The CanAg Free PSA EIA (Prod. No. 350-10) was compared to the two-step CanAg Free PSA EIA (330-10). One hundred twenty-seven male human serum samples ranging in values from 0–9 µg/L were measured and linear regression analyses of the results yielded:

$$[\text{Free PSA}]_{\text{Prod. No. 350-10}} = 1.02 \times [\text{Free PSA}]_{\text{Prod. No. 330-10}} - 0.06 \qquad r = 0.99$$

WARRANTY

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

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CanAg Free PSA EIA Prod. No. 350-10, 2013-09, F5679, r6