

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<sub>0197</sub>

## 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 οδηγιών 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 ОБОЗНАЧЕНИЯ



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

LOT

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



Date of manufacture/  
Herstellungsdatum/  
Fecha de fabricación/  
Data di fabbricazione/  
Date de fabrication/  
Produktie datum/Produktionsdato/  
Datum výroby/Ημερομηνία  
Παράγωγής/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/  
Katalognummer/Katalogové číslo/  
Αριθμός καταλόγου/  
Referència de catálogo/  
Katalógusszám/Produktnummer/  
Numer katalogowy/Katalogo numeris/  
Номер по каталогу



Manufacturer/Hersteller/Fabricante/  
Fabbicante/Fabrant/Fabrikant/  
Producent/Výrobce/Κτασκευαστής/  
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/Ineholder 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



In Vitro Diagnostic Medical Device/  
In Vitro Diagnostikum/Producto  
sanitario para diagnóstico in vitro/  
Dispositivo médico-diagnóstico 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  
πρωτόν/Dispositivo médico para  
diagnóstico in vitro/In vitro  
diagnostikum/Endast för in vitro-  
diagnostik/Wyrób do diagnostyki In  
Vitro/In Vitro Diagnostinė Medicinos  
Priemonė/Только для диагностики  
In Vitro



Temperature limitation/  
Temperaturbegrenzung/  
Limite de temperatura/  
Limiti di temperatura/  
Limites de température/  
Temperatuurlimiet/  
Temperaturbegrænsning/  
Teplotní rozmezi od do/  
Περιορισμοί θερμοκρασίας/  
Limites de temperatura/  
Hőmérséklettartomány/  
Temperaturbegrænsning/  
Przestrzegać zakresu temperatury/  
Temperatūriņai 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/Néze meg a Használati  
utasítást/Se bruksanvisning/Sprawdź  
w instrukcji obsługi/Dėl naudojimo  
žūrėkite instrukcijas/  
Обратитесь к инструкции по  
применению



Biological risks/Biogegefährdung/  
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

ORIG MOU

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

ORIG HUM

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

CONT

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

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## WARNINGS AND PRECAUTIONS

### For in vitro diagnostic use

GB

- 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.

### Caution

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV 1 and 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.

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## WARNHINWEISE UND VORSICHTSMASSNAHMEN

### Für In-vitro-Diagnostik

DE

- 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.

### Achtung

Das zur Herstellung der Reagenzien aus humaner Quelle verwendete Material wurde auf HIV-1/2-Antikörper, HCV-Antikörper und Hepatitis-B-Oberflächenantigen (HBsAg) getestet und als nicht reaktiv befunden. Da es keine Methode gibt, mit der das Vorliegen von durch Blut übertragenen Krankheiten vollkommen ausgeschlossen werden kann, sollten der Umgang mit Reagenzien aus humaner Quelle und deren Entsorgung so erfolgen, als handele es sich um potenziell infektiöses Material.

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## 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) publicación 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.

### Precaución

Material usado en la preparación de este reactivo se analizó la presencia de anticuerpos HIV 1 y 2, anticuerpos HCV y antígenos de superficie de hepatitis B, siendo el resultado de dichos análisis negativo. Sin embargo, como el test no puede excluir completamente los anticuerpos HIV 1 y 2, anticuerpos HCV y antígenos de superficie de hepatitis B, el manejo y disposición del reactivo debe ser como potencialmente infecciosas.

## 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

### Precauzioni

Le sostanze usate nella preparazione dei reagenti sono state testate e trovate Non Reattive per l'anticorpo anti-HIV 1 e 2, per l'anticorpo anti-HCV e l'antigene di superficie dell'Epatite B (HbsAg). Tuttavia poiché nessun metodo diagnostico è in grado di escludere completamente la possibilità di trasmissione di infezioni attraverso il sangue si consiglia di maneggiare questi reattivi come potenzialmente infettivi.

## PRÉCAUTIONS D'EMPLOI ET MISE EN GARDE

FR

### Pour un usage diagnostique in Vitro

- Pour usage professionnel seulement.
- Prière de se référer à la Publication N° : (CDC) 88-8395 de l'U.S. Département of Health and Human Services (Bethesda, Md., USA) sur les procédures de sécurité dans les laboratoires ou toutes autres réglementations locales et nationales.
- Manipuler les échantillons de patients comme potentiellement infectieux.
- Suivre les réglementations locales pour l'élimination et le traitement de tous les déchets.

### Attention

Le matériel utilisé pour la préparation de réactifs d'origine humaine, a été testé et trouvé non réactif aux Anticorps anti-virus de l'immunodéficience humaine (VIH-1/2), aux Anticorps de l'Hépatite C (VHC) et à l'Antigène de surface de l'Hépatite B (AgHBs). Puisqu'il n'existe pas de méthode de test, rejetant complètement la présence de maladies dans le sang, la manipulation et l'élimination de réactifs d'origine humaine doivent être effectuées comme s'ils étaient potentiellement infectieux.

## 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.

### Advarsel

Alt materiale anvendt ved beredningen af reagenser af human oprindelse er blevet testet og fundet negative for HIV 1 og 2 antistoffer, HCV antistoffer og Hepatitis B overflade antigen (HbsAg). Da ingen analysemetoder fuldstændig kan udelukke tilstedeværelsen af blodbårne sygdomme, skal håndtering og bortskaffelse af reagenser af human oprindelse fra dette produkt behandles som potentielt smittefarligt.

## ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ ΚΑΙ ΠΡΟΦΥΛΑΞΕΙΣ

GR

### Για *in vitro* διαγνωσική χρήση

- Για επαγγελματική χρήση, μόνο.
- Παρακαλούμαι όπως επικαλεστείτε τις οδηγίες ασφαλούς λειτουργίας των εργαστηρίων του Τμήματος Υγείας και Ανθρώπινων 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.

### Προσοχή

Όλα τα υλικά που χρησιμοποιούνται για την παρασκευή αντιδραστηρίων ανθρώπινης προέλευσης έχουν εξετασθεί και έχουν βρεθεί αρνητικά για HIV-1/2 Αντίσωμα (Ab), HCV Αντίσωμα (Ab) και Ηπατίτιδας Β Αντιγόνο Επιφανείας (Hepatitis B Surface Antigen) (HBsAg). Εφ'όσον δεν υπάρχει μέθοδος ικανή να αποκλείσει απόλυτα την παρουσία αιματολογικών / μολυσματικών ασθενειών, ο τρόπος μεταχείρισης και η απομάκρυνση αντιδραστηρίων ανθρώπινης προέλευσης αυτού του συγκεκριμένου προϊόντος, πρέπει να είναι ίδιος με αυτόν που ακολουθείται για μολυσμένα δείγματα.

## 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.

### Varning

Material som använts för tillverkning av reagens med humant ursprung har testats och befunnits negativt för HIV 1 och 2 antikroppar, HCV antikroppar samt hepatit B ytantigen (HBsAg). Eftersom inget test fullständigt kan utesluta ev. närvaro av blodsmitta skall hantering och bortskaffande av humant material från denna produkt ske som om den vore potentiellt infektiös.

# 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  $\alpha_1$ -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
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### MICROPLA

<b>Microplate</b>	1 Plate	2–8°C until expiry date stated on the plate
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12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

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

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

<b>CONJ</b>	<b>Anti-FPSA</b>
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<b>Tracer, HRP Anti-Free PSA</b>	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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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.

<b>SUBS</b>	<b>TMB</b>
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<b>TMB HRP-Substrate</b>	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.

Component	Quantity	Storage and stability after first opening
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**STOP**

<b>STOP Solution</b>	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**

<b>Wash Concentrate</b>	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

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- 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  $\geq$  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

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### Materials required but not supplied with the kit

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#### 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  $\mu$ L/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 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 resenser pipette with disposable plastic tips for delivery of 100  $\mu$ L is useful but not essential.

#### 5. Distilled or deionized water

For preparation of Wash Solution.

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## Procedural notes

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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	Bottle/Plate	Procedure																											
1. Prepare wash solution	<span style="border: 1px solid black; padding: 2px;">WASHBUF</span> <span style="border: 1px solid black; padding: 2px;">25X</span>	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.																											
	<span style="border: 1px solid black; padding: 2px;">CONJ</span> <span style="border: 1px solid black; padding: 2px;">Anti-FPSA</span>																												
	<span style="border: 1px solid black; padding: 2px;">BIOTIN</span> <span style="border: 1px solid black; padding: 2px;">Anti-FPSA</span>																												
Prepare Antibody solution		Mix 50 $\mu$ L of Tracer, HRP Anti-Free PSA, with 1 mL of Biotin Anti-Free PSA per strip:																											
		<table border="1"><thead><tr><th>No. of Strips</th><th>Tracer, HRP Anti-Free PSA (<math>\mu</math>L)</th><th>Biotin Anti-Free PSA (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></tbody></table>	No. of Strips	Tracer, HRP Anti-Free PSA ( $\mu$ L)	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
No. of Strips	Tracer, HRP Anti-Free PSA ( $\mu$ L)	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	<b>MICROPLA</b>	Wash each well once with wash solution				
3.	Add calibrators, controls and samples	<b>CAL</b>	<b>PSA</b>	50 $\mu$ L in each well			
		0, 0.3, 1, 2, 5, 10					
		<b>CONTROL</b>	<b>FPSA</b>				
		1, 2					
4.	Add Antibody solution	<b>ANTIBODY SOLUTION</b>					
		100 $\mu$ L in each well					
5.	Incubate	<b>MICROPLA</b>					
		1 hour shaking at room temperature					
6.	Wash	<b>MICROPLA</b>					
		Wash each well six times with wash solution					
7.	Add TMB HRP-Substrate	<b>SUBS</b>	<b>TMB</b>	100 $\mu$ L in each well			
8.	Incubate	<b>MICROPLA</b>					
		30 min shaking at room temperature					
9.	Add Stop Solution	<b>STOP</b>					
		100 $\mu$ L in each well					
10.	Incubate	<b>MICROPLA</b>					
		1 min shaking at room temperature					
11.	Read absorbance	<b>MICROPLA</b>					
		Read at 450 nm within 15 min.					

Preparation of reagents	Stability of prepared reagent
<b>Wash Solution</b>	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.

<b>Antibody Solution</b>	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.

- 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.
- Pipette 50  $\mu\text{L}$  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					

- Add 100  $\mu\text{L}$  of Antibody Solution to each well using a 100  $\mu\text{L}$  precision pipette (or an 8-channel 100  $\mu\text{L}$  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.
- Incubate the frame containing the strips for 1 hour ( $\pm 10$  min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
- Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.
- Add 100  $\mu\text{L}$  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.
- Incubate for 30 min ( $\pm 5$  min) at room temperature with constant shaking. Avoid direct sunlight.

9. Add 100  $\mu\text{L}$  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  $\mu\text{g/L}$ . 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 1<sup>st</sup> 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 1<sup>st</sup> 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:

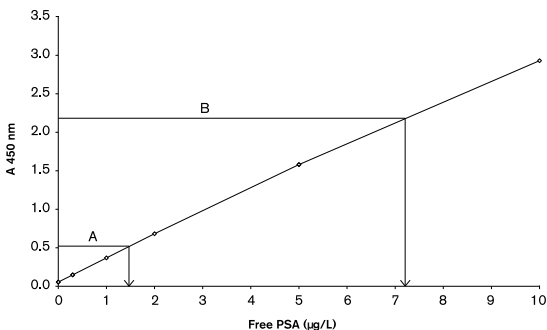
- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0  $\mu\text{g/L}$ .
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0  $\mu\text{g/L}$ .
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0  $\mu\text{g/L}$ .

**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  $\mu\text{g/L}$ ), 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 ( $\mu\text{g/L}$ )
CAL	PSA	0	0 $\mu\text{g/L}$	0.054	
CAL	PSA	0.3	0.3 $\mu\text{g/L}$	0.148	
CAL	PSA	1	1 $\mu\text{g/L}$	0.369	
CAL	PSA	2	2 $\mu\text{g/L}$	0.683	
CAL	PSA	5	5 $\mu\text{g/L}$	1.580	
CAL	PSA	10	10 $\mu\text{g/L}$	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:

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

### 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 prostatic 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)			Clinical sensitivity (PCa ≤ cut-off)		
	n	%	(95% confidence interval)	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:

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

### 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  $\pm 10\%$  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 ( $\pm 10\%$ ) 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:

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

**WARRANTY**

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by 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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