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

CanAg CA15-3 EIA

REF 200-10

IVD

CE

Instructions for use. 2009-06

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

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

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

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

DK **Kontakt venligst den danske distributør for gældende version af dansk brugsanvisning.**

GR **Παρακαλούμε όπως επικοινωνήσετε με τον προμηθευτή σας για την έγκυρη απόδοση στα Ελληνικά των οδηγιων χρήσης**

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

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

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

LOT

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

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

REF

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

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

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

IVD

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

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

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

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

CONT

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

ORIG | MOU

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

ORIG | HUM

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

WARNINGS AND PRECAUTIONS

GB

For in vitro diagnostic use

- For Professional Use Only
- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88–8395 on laboratory safety procedures or any other local or national regulation.
- Handle all patient specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

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.

WARNHINWEISE UND VORSICHTSMASSNAHMEN

DE

Für In-vitro-Diagnostik

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

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.

CUIDADOS Y PRECAUCIONES

ES

Para diagnóstico in vitro

- Solo para uso profesional
- Consultar la publicación del U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88–8395 o las normas locales o nacionales.
- Tratar todas las muestras de pacientes como potencialmente infecciosas.
- Todos los residuos se deben tirar cumpliendo las normas en vigor.

Precaución

Material usado en la preparación de este reactivo se analizó la presencia de anticuerpos HIV 1 y 2, anticuerpos HCV y antigenos 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 antigenos 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 poichè 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 diagnostic in Vitro

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

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 λειτουγίας των εργαστηρίων του Τμήματος Υγείας και Ανθρωπινων Υπηρεσιών των Η.Π.Α.(U.S. Department of Health and Human Services) (Bethesda, Md., USA) αριθμός έκδοσης (CDC) 88—8395, ή οποιοδήποτε άλλο κατά τόπους σχετικό Εθνικό κανονισμό.
- Μεταχειριστήτε όλα τα δείγματα ως μολυσμένα.
- Ακολουθείστε τις κατά τόπου οδηγίες για απομάκρυνση άχρηστου υλικού.

Προσοχή

Ολα τα υλικά που χρησιμοποιούται 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 Επιφανειας (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 προϊοντος, 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.

GB

CanAg CA15-3 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CA15-3 EIA kit is intended for the quantitative determination of the cancer associated antigen MUC-1 (CA15-3) in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The MUC-1 antigen is a membrane-anchored mucin-type glycoprotein present in malignant and normal epithelial cells of certain organs, e.g. breast, lung, ovary, pancreas and colon (1). The apoprotein of the MUC-1 mucin contains a transmembrane domain, a cytoplasmic domain, and an extracellular carbohydrate rich domain. The extracellular domain is characterized by polymorphism with respect to the number of a 20 amino acid tandem repeat (VNTR polymorphism). The CanAg CA15-3 EIA is based on two mouse monoclonal antibodies, Ma695 as catcher antibody recognizing a sialylated carbohydrate epitope expressed on the MUC-1 antigen and Ma552 as tracer antibody targeting the PD**TRPAP**G region of the protein core (2-5).

The MUC-1 breast cancer mucin (CA15-3 antigen) is secreted from tumor cells and is a well-established serological marker for monitoring the clinical course of breast cancer patients (6).

PRINCIPLE OF THE TEST

The CanAg CA15-3 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-CA15-3 monoclonal antibody and horseradish peroxidase (HRP) labelled Anti-CA15-3 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 CA15-3 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 CA15-3 concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA15-3 EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8°C. Do not freeze.
- Opened reagents are stable accordning to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first opening
MICROPLA		
Microplate	1 Plate	2–8°C until expiry date stated on the plate

12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminium pouch containing desiccant and reseal carefully to keep dry.

CA15-3 Calibrators			5 vials	2–8°C until expiry date stated on the vials
CAL	**CA15-3**	**0**	0 U/mL 1 x 0.75 mL	
CAL	**CA15-3**	**15**	15 U/mL 1 x 0.75 mL	
CAL	**CA15-3**	**50**	50 U/mL 1 x 0.75 mL	
CAL	**CA15-3**	**125**	125 U/mL 1 x 0.75 mL	
CAL	**CA15-3**	**250**	250 U/mL 1 x 0.75 mL	

MUC-1 antigen 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
CA15-3 Controls	2 vials	2–8°C until expiry date stated on the vials
CONTROL CA15-3 1	1 x 0.75 mL	
CONTROL CA15-3 2	1 x 0.75 mL	

MUC-1 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

BIOTIN Anti-CA15-3

Biotin Anti-CA15-3	1 x 15 mL	2–8°C until expiry date stated on the vial

Biotin Anti-CA15-3 monoclonal antibody from mouse, approximately 2.5 µg/mL. Contains phosphate buffered saline (pH 7.2) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergents, an inert blue dye and 0.01% methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-CA15-3 before use.

CONJ Anti-CA15-3

Tracer, HRP Anti-CA15-3	1 x 0.75 mL	2–8°C until expiry date stated on the vial

Stock solution of HRP Anti-CA15-3 monoclonal antibody from mouse, approximately 50 µg/mL. Contains preservatives. To be mixed with Biotin Anti-CA15-3 before use.

DIL SPE

Sample Diluent	2 x 50 mL	2–8° C until expiry date stated on the bottle

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

Additional Sample Diluent can be obtained by ordering the product: “Sample Diluent CA15-3 EIA” Prod. No. 200-24 containing 50 mL.

Component	Quantity	Storage and stability after first opening
SUBS TMB		
TMB HRP-Substrate	1 x 12 mL	2–8°C until expiry date stated on the vial
Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.		
STOP		
STOP Solution	1 x 15 mL	2–8°C until expiry date stated on the vial
Contains 0.12 M hydrochloric acid. Ready for use.		
WASHBUF 25X		
Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with water 25 times before use.		

Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

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

Caution

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SPECIMEN COLLECTION AND HANDLING

The CanAg CA15-3 EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8°C for 24 hours. For longer periods store samples at -70°C or below. Tubes containing gel should not be used for long-term storage. Samples should not be stored in a self-defrosting freezer and not be thawed and refrozen before analysis. Allow frozen samples to thaw slowly at 2–8°C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 700-900/min.
2. **Microplate wash device**
   Automatic plate wash capable of performing 1 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.
   The Nunc Immuno-8 manual strip washer is recommended if an automatic 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 preparation of Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg CA15-3 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.

Protocol Sheet

CanAg CA15-3 EIA REF 200-10

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

Step	Bottle/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-CA15-3 BIOTIN Anti-CA15-3	Mix 50 µL of Tracer, HRP Anti-CA15-3, with 1 mL of Biotin Anti-CA15-3 per strip:

No. of Strips	Tracer, HRP Anti-CA15-3 (µL)	Biotin Anti-CA15-3 (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. Sample Diluent	DIL SPE	Mix 25 µL of patient sample with 1 mL of Sample Diluent.	
3. Wash	MICROPLA	Wash each well once with wash solution	
4. Add calibrators, controls and diluted samples	CAL CA15-3 0, 15, 50, 125, 250 CONTROL CA15-3 1, 2	25 µL in each well	
5. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well	
6. Incubate	MICROPLA	2 hours shaking at room temperature	
7. Wash	MICROPLA	Wash each well six times with wash solution	
8. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well	
9. Incubate	MICROPLA	30 min shaking at room temperature	
10. Read absorbance	MICROPLA	620 nm	
Alt. 10 Add Stop Solution	STOP	100 µL in each well	
Alt. 11 Incubate	MICROPLA	1 min shaking at room temperature	
Alt. 12 Read absorbance	MICROPLA	Read at 405 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 in a sealed container

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-CA15-3 with 1 mL of Biotin Anti-CA15-3 per strip (see table below and the Protocol Sheet).

No. of Strips	Tracer, HRP Anti-CA15-3 (µL)	Biotin Anti-CA15-3 (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-CA15-3 into the vial of Biotin Anti-CA15-3 and mix gently. Make sure that all of the Tracer, HRP Anti-CA15-3 is transferred to the vial of Biotin Anti-CA15-3.

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. Dilute serum samples 1:41 by mixing 25 µL of sample with 1 mL of Sample Diluent. **NOTE:** CA15-3 Calibrators and CA15-3 Controls 1 and 2 shall **not** be diluted.
3. Transfer the required number of microtrips 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.
4. Pipette 25 µL of the CA15-3 Calibrators (CAL 0, 15, 50, 125, 250), Controls (C1, C2) and diluted 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 250	Unk 2				
B	Cal 0	Cal 250	Unk 2				
C	Cal 15	C1	etc.				
D	Cal 15	C1					
E	Cal 50	C2					
F	Cal 50	C2					
G	Cal 125	Unk 1					
H	Cal 125	Unk 1					

5. 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.

6. Incubate the frame containing the strips for 2 hours (± 5 min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
7. Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.
8. Add 100 µL of TMB HRP-Substrate to each well using the same pipetting procedure as in item 5. 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.
9. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
10. 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. 10. Add 100 µL of Stop Solution. Mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 minutes after addition of Stop Solution.

Measurement range

The CanAg CA15-3 EIA measures concentrations between 1 and 250 U/mL. If CA15-3 concentrations above the measuring range are to be expected, it is recommended to dilute samples 1/400 and 1/4000 with Sample Diluent prior to analysis.

Quality control

CA15-3 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 also 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

Since no common reference material is available for the MUC-1 (CA15-3) antigen, CanAg CA15-3 EIA 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 CA15-3 Calibrators.
For automatic calculation of CA15-3 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 U/mL.
- 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 U/mL.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.

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 CA15-3 calibrator against the corresponding CA15-3 concentration (in U/mL), see figure below. The unknown CA15-3 concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

If samples in an initial analysis give CA15-3 levels higher than 250 U/mL the diluted samples (1/41) should be further diluted 1/10 and 1/100 with Sample Diluent to obtain the accurate CA15-3 concentration of the samples.

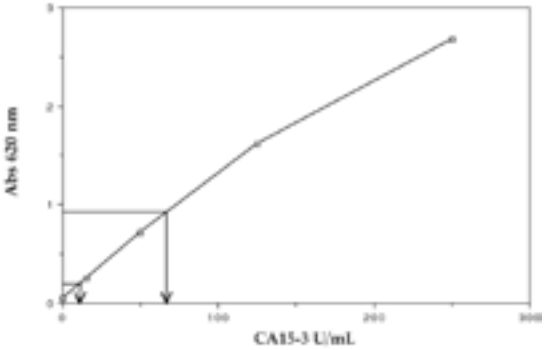
1/10 dilution = 50 µL of specimen + 450 µL of Sample Diluent
1/100 dilution = 50 µL of 1:10 dilution + 450 µL of Sample Diluent

The CA15-3 concentration of the undiluted sample is then calculated as:

Dilution 1/10: 10 x measured value
Dilution 1/100: 100 x measured value

Example of results

Specimen	Calibrator values	Mean abs value (A)	CA15-3 (U/mL)
CAL CA15-3 0	0 U/mL	0.044	
CAL CA15-3 15	15 U/mL	0.252	
CAL CA15-3 50	50 U/mL	0.723	
CAL CA15-3 125	125 U/mL	1.612	
CAL CA15-3 250	250 U/mL	2.680	
Specimen A		0.241	14.1
Specimen B		0.895	63.1



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

LIMITATIONS OF THE PROCEDURE

The level of CA15-3 cannot be used as absolute evidence for the presence or absence of malignant disease and the CA15-3 test should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the management of patients, and the CA15-3 test should not replace any established clinical examination.

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 buffers.

EXPECTED VALUES

CanAg CA15-3 was measured in 51 healthy female blood donors. The mean value obtained was 15 U/mL with a standard deviation of 6.8. The median value was 13.8 U/mL, range 6– 36 U/mL. The lower and upper extremes of the normal range were examined using IFCC recommended non-parametric statistical treatment. The reference interval contains the central 95% fraction of the reference distribution. Reference limits may accordingly be estimated as the 2.5% (lower) and 97.5% (upper) fractiles. These limits cut off a fraction of 2.5% of the values in each tail of the reference distribution. Non-parametric estimates:
N=51

Fraction	Reference limit (U/mL)
2.5th (lower)	7
97.5th (upper)	36

94% of the healthy women had assay values below 30 U/mL.

It is recommended that each laboratory establish their own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc. It should also be borne in mind that the individual patient's own baseline results provides the most important reference point for interpretation of marker results.

PERFORMANCE CHARACTERISTICS

Precision

Total precision was calculated according to NCCLS guideline EP5-A (7) using four levels of frozen pooled human serum containing added human CA15-3. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days. The analyses were undertaken during a period of 40 months by ≥ three different technicians and using 20 different CanAg CA15-3 EIA reagent combinations.

Sample	Replicates	Mean (U/mL)	Within-run SD (U/mL)	Within-run CV %	Between-day SD (U/mL)	Between-day CV %
CA15-3 1	80	15.8	0.55	3.5	1.16	7.3
CA15-3 2	80	57.0	1.73	3.0	6.37	11
CA15-3 3	80	78.6	2.93	3.7	5.29	6.7
CA15-3 4	80	148	4.86	3.3	8.37	5.6

Detection limit

The detection limit of the CanAg CA15-3 EIA is < 1 U/mL defined as the concentration corresponding to the mean of the absorbance values of the CA15-3 calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 15 - OD\ CAL\ 0} \times 15\ U/mL$$

Recovery

Spiked serum samples were prepared by adding human CA15-3 antigen to normal serum samples. The recovery of the added antigen was in the range 95–110 %.

Hook effect

No hook effect has been noticed with samples up to 7 500 U/mL. **NOTE:** In very high samples the colour of the substrate will change from blue to greenish (and eventually yellow in extremely high samples). This will lead to a falsely low absorbance at 620 nm, and in extreme cases the absorbance may fall within the calibration curve range and noticed as a hook.

Linearity

Patient samples were serially diluted with Sample Diluent and analysed. The obtained values were between 93–102 % of the expected values in the range 25–250 U/mL.

Specificity

The CanAg CA15-3 EIA is based on two mouse monoclonal antibodies, the catching MAb Ma695 targeting a sialylated carbohydrate epitope and the detecting MAb Ma552 targeting the PD**TRPAP**G hydrophilic peptide of the protein core. The NCCLS guideline EP7-P (8) was followed to determine possible sources of interference. The following substances and concentrations were tested and found not to interfere with the test.

	Concentration with no significant (± 10%) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	5 mg/mL

WARRANTY

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

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