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

CanAg CA242 EIA

REF 101-10

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/Bioloģinis pavojus/
Биологическая опасность

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

CONT

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

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.
- Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
- 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.
- Die Reagenzien enthalten Natriumazid (NaN_3) als Konservierungsmittel. Natriumazid kann mit Blei- und Kupferleitungen reagieren und hochexplosive Metallazide bilden. Spülen Sie die Leitungen beim Wegschüt ten mit viel Wasser, um einer Azidbildung vorzubeugen.
- 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.
- Los reactivos contienen azida sódica (NaN_3) come conservante. La azida sódica puede reaccionar con el plomo o el cobre de las tuberías, formando azidas metálicas muy explosivas. Al limpiar los reactivos, dejar correr gran cantidad de agua para evitar la formación de azidas.
- 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
- I reattivi contengono sodio azide (NaN_3) come conservante. Il sodio azide può reagire con piombo e rame formando azidi metallici altamente esplosivi. Quando i reattivi vengono scartati lavare con abbondante quantità di acqua per prevenire il rischio di reazione dell'azide
- Seguire le normative vigenti relative all'eliminazione del materiale usato

Precauzioni

Le sostanze usate nella preparazione di reattivi di origine umana sono state testate e trovate Non Reattive per l'anticorpo anti-HIV 1/2, l'anticorpo anti-HCV e per l'antigene di superficie dell'Epatite B (HBsAg). Tuttavia poichè nessun metodo diagnostico è in grado di escludere completamente patologie correlate alla presenza di questi anticorpi ed antigeni, la manipolazione e lo scarto dei reattivi di origine umana di questo prodotto, deve essere effettuata come se essi fossero 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.
- Réactifs contenant de l'Azide de Sodium (NaN3) comme conservateur: l'Azide de Sodium peut réagir avec les tubes en plomb et en cuivre pour former des Azides de métaux hautement explosifs. Lors de l'élimination, répandre une grande quantité d'eau pour prévenir la formation des Azides.
- Suivre les réglementations locales pour l'élimination et le traitement de tous les déchets.

Attention

Le materiel 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.
- Reagenser indeholder natriumazid som præserveringsmiddel. Natriumazid kan danne eksplosive syrer i metalafløb. Anvend korrekt affaldsprocedure.
- 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.
- Αποφύγετε επαφή με αντιδραστήρια που περιέχουν υπεροξείδιο του υδρογόνου ή υδροχλωρικό οξύ. Σε περίπτωση επαφής με τέτοιου είδους αντιδραστήρια, πλυθείτε σχολαστικά με άφθονο νερό.
- 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.
- Vissa reagens innehåller natriumazid (NaN_3) som konserveringsmedel. Natriumazid kan reagera med bly- och kopparledningar och bilda explosiva metall-azider. Använd rikligt med vatten vid nedspolning i avloppet för att förhindra metall-azid bildning.
- 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 bortförskaffande av humant material från denna produkt ske som om den vore potentiellt infektiös.

GB

CanAg CA242 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CA242 EIA kit is intended for the quantitative determination of CA242 cancer antigen in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The tumor marker CA242 is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant have been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated Lewis^a (CA19-9). Thus, CA242 is related, but not identical to the epitope of CA19-9 (1, 2). Serum levels of CA242 are low in healthy subjects and subjects with benign diseases, while elevated levels are commonly found in serum from patients with gastro-intestinal cancer (3).

The CA242 marker may be used as an aid in the diagnosis and management of patients with known or suspected gastro-intestinal carcinomas (4-9). The CanAg CA242 EIA should not be used as a substitute for any established clinical examination of malignancy, but may be used as a complement to existing clinical and laboratory methods.

PRINCIPLE OF THE TEST

The CanAg CA242 EIA is a solid-phase, non-competitive immunoassay based on the direct sandwich technique. Calibrators, controls and patient samples are incubated together with biotinylated anti-CA242 monoclonal antibody (MAb) C241 in Streptavidin coated microstrips. CA242 present in calibrators, controls or samples is adsorbed to the Streptavidin coated microstrips by the biotinylated anti-CA242 MAb during the incubation. The strips are then washed and incubated with horseradish peroxidase (HRP) labelled Anti-CA242 MAb C242. 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 CA242 antigen 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 CA242 concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA242 EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8°C. Do not freeze.
- Opened reagents are stable accordning to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

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

12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

CA242 Calibrators			5 vials	2–8°C until expiry date stated on the vials
CAL	CA242	0	0 U/mL 1 x 0.75 mL	
CAL	CA242	15	15 U/mL 1 x 0.75 mL	
CAL	CA242	50	50 U/mL 1 x 0.75 mL	
CAL	CA242	100	100 U/mL 1 x 0.75 mL	
CAL	CA242	150	150 U/mL 1 x 0.75 mL	

Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye, and 0.05% NaN_3 as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
CA242 Controls	2 vials	2–8°C until expiry date stated on the vials
CONTROL CA242 1	1 x 0.75 mL	
CONTROL CA242 2	1 x 0.75 mL	

Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.05% NaN_3 as preservative. Ready for use.

BIOTIN Anti-CA242		
Biotin Anti-CA242	1 x 15 mL	2–8°C until expiry date stated on the vial

Biotin Anti-CA242 monoclonal antibody from mouse, approximately 1.5 µg/mL. Contains Tris-HCl buffered saline (pH 7.75) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert red dye and 0.05% NaN_3 as preservative. Ready for use.

CONJ Anti-CA242		
Tracer, HRP Anti-CA242	1 x 0.75 mL	2–8°C until expiry date stated on the vial

Stock solution of HRP Anti-CA242 monoclonal antibody from mouse, approximately 40 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL CONJ		
Tracer Diluent	1 X 15 mL	2–8° C until expiry date stated on the vial

Phosphate buffered saline (pH 7.2) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert blue dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

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

Indications of instability

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

WARNINGS AND PRECAUTIONS

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- Handle all patient specimens as potentially infectious.
- Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
- 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

The CanAg CA242 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 it is recommended to store the samples at –20°C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 700-900/min.
2. **Microplate wash device**
   Automatic plate wash capable of performing 1, 3 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 CA242 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 (20–28°C) prior to use. The assay should only be performed at temperatures between 20–28°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 CA242 EIA REF 101-10

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 or deionized water.
	Prepare Tracer working solution	CONJ | Anti-CA242 DIL | CONJ	Mix 50 µL of Tracer, HRP Anti-CA242 with 1mL of Tracer Diluent per strip:

No. of Strips	Tracer, HRP Anti-CA242 (µL)	Tracer Diluent (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. Use manual or automatic washer.

3. Add calibrators, controls and samples	CAL | CA242 0, 15, 50, 100, 150 CONTROL | CA242 1, 2	25 µL in each well
4. Add Biotin Anti-CA242	BIOTIN | Anti-CA242	100 µL in each well
5. Incubate	MICROPLA	2 hours shaking at room temperature
6. Wash	MICROPLA	Wash each well three times with Wash Solution. Use manual or automatic washer.
7. Add Tracer working solution	TRACER WORKING SOLUTION	100 µL in each well
8. Incubate	MICROPLA	1 hour shaking at room temperature
9. Wash	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.
10. Add TMB HRP-Substrate	SUBS | TMB	100 µL in each well
11. Incubate	MICROPLA	30 min shaking at room temperature
12. Read absorbance	MICROPLA	620 nm
Alt.12 Add Stop Solution	STOP	100 µL in each well
Alt.13 Incubate	MICROPLA	1 min shaking at room temperature
Alt.14 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.

Tracer Working Solution	3 weeks at 2–8°C in a sealed container

Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-CA242 with 1 mL of Tracer Diluent per strip (see table below):

No. of Strips	Tracer, HRP Anti-CA242 (µL)	Tracer Diluent (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 Tracer Working Solution.

Alternative: Pour the content of the Tracer, HRP Anti-CA242 into the vial of Tracer Diluent and mix gently. Make sure that all of the Tracer, HRP Anti-CA242 is transferred to the vial of Tracer Diluent.

NOTE: The Tracer Working Solution is stable for 3 weeks at 2–8°C. Do not prepare more Tracer Working 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 (20–28°C) before use.

1. Start to prepare Wash Solution and Tracer Working Solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 25 µL of the CA242 Calibrators (CAL 0, 15, 50, 100, 150), Controls (C1, C2) 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 150	Unk 2				
B	Cal 0	Cal 150	Unk 2				
C	Cal 15	C1	etc.				
D	Cal 15	C1					
E	Cal 50	C2					
F	Cal 50	C2					
G	Cal 100	Unk 1					
H	Cal 100	Unk 1					

4. Add 100 µL of Biotin Anti-CA242 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 2 hours (± 10 min) at room temperature (20–28°C) with constant shaking of the plate using a microplate shaker.
6. After the first incubation aspirate and wash each strip 3 times using the wash procedure described in Procedural notes, item 4.

7. Add 100 µL of Tracer working solution to each well. Use the same pipetting procedure as in item 4 above.
8. Incubate the frame for 1 hour (± 5 min) at room temperature (20–28°C) with constant shaking.
9. After the second incubation aspirate and wash each strip 6 times, using the wash procedure described in Procedural notes, item 4.
10. 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.
11. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
12. 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. 12. 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 CA242 EIA measures concentrations between 1 and 150 U/mL. If CA242 concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human serum prior to analysis. **NOTE**: The serum used for dilution must also be measured in order to determine the endogenous CA242 concentration (see "Calculation of results").

Quality control

CA242 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

Since no common reference material is available for CA242 antigen, CanAg CA242 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 CA242 Calibrators.
For automatic calculation of CA242 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 CA242 Calibrator against the corresponding CA242 concentration (in U/mL), see figure below. The unknown CA242 concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

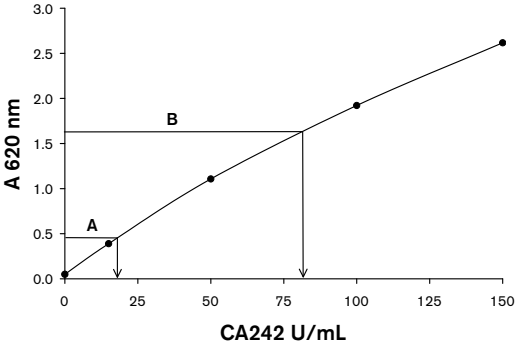
If samples in an initial analysis give CA242 levels higher than 150 U/mL, the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate CA242 concentration. **NOTE**: The sample used for dilution must also be measured in order to determine the endogenous CA242 concentration.

The CA242 concentration of the undiluted sample is calculated as:

Dilution 1/10: $10 \times ([CA242]_{\text{Diluted sample}} - (0.9 \times [CA242]_{\text{Normal serum}}))$

Example of results

Specimen	Calibrator values	Mean abs value (A)	CA242 (U/mL)
CAL CA242 0	0 U/mL	0.050	
CAL CA242 15	15 U/mL	0.390	
CAL CA242 50	50 U/mL	1.107	
CAL CA242 100	100 U/mL	1.922	
CAL CA242 150	150 U/mL	2.617	
Specimen A		0.410	16.1
Specimen B		1.636	80.9



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

LIMITATIONS OF THE PROCEDURE

The level of CA242 cannot be used as absolute evidence for the presence or absence of malignant disease, and the CA242 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 CA242 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 buffer.

EXPECTED VALUES

Serum samples from 184 apparently healthy blood donors, 97 women and 87 men were analysed resulting in a mean value of 8.5 $\pm$ 7.6 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:

Fraction	Reference limit (U/mL)	95% confidence interval
2.5th (lower)	0	0–0
97.5th (upper)	29	25–44

93% of the healthy subjects had assay values $\leq$ 20 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 result provides the most important reference point for interpretation of marker results (9).

PERFORMANCE CHARACTERISTICS

Precision

Total precision was determined according to NCCLS guideline EP5-A (10) using four levels of frozen pooled human serum containing added CA242 from patients with gastro-intestinal cancer. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days. The analyses were undertaken during a period of 42 months, by ≥ two different technicians and using 20 different CanAg CA242 EIA kit batches.

Sample	Replicates	Mean (U/mL)	Within-run SD (U/mL)	Within-run CV %	Between-day SD (U/mL)	Between-day CV %
CA242 1	80	16.2	0.67	4.1	0.39	2.4
CA242 2	80	48.4	1.93	4.0	1.82	3.8
CA242 3	80	79.5	2.99	3.8	2.46	3.1
CA242 4	80	125	5.81	4.7	2.74	2.2

Detection limit

The detection limit of the CanAg CA242 EIA is < 1 U/mL defined as the concentration corresponding to the mean of the absorbance values of the CA242 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 CA242 antigen to normal serum samples. The recovery of the added antigen was in the range 87 –107 %. **NOTE:** recovery studies should **not** be performed using the kit calibrators.

Hook effect

No hook effect has been noticed with samples up to 150 000 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 normal lipid stripped human serum and analysed. The obtained values were in the range 97–108%.

Specificity

The CanAg CA242 EIA is based on two mouse monoclonal antibodies, the catching MAb C241 targeting sialylated Lewis^a and the detecting MAb C242 specific for the CA242 epitope. Thus the assay determines S-Le^a containing mucin antigen(s) expressing the CA242 epitope. The NCCLS guideline EP7-P (11) 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®)	8 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	5 mg/mL

Method comparison

The CanAg CA242 EIA was compared to the CA242 Delfia. 145 serum samples from healthy blood donors and from patients with malignant and non-malignant diseases, ranging in values from 0-250 U/mL were analysed and linear regression analyses of the results yielded (4):

CanAg CA242 EIA = 1.02 x CA242 Delfia – 1.1 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 event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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Fujirebio Diagnostics AB
Elof Lindälvs gata 13
PO Box 121 32
SE-402 42 Göteborg
Sweden
Phone + 46 31 85 70 30
Fax + 46 31 85 70 40
info@fdab.com
www.fdab.com

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