

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



CanAg CA19-9 EIA

REF

120-10

IVD

CE

Instructions for use. 2008-12

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 ОБЪЯСНЕНИЯ



Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Houdbaar tot/Holdbar til/
Ρουζιτηλέ do/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 nummer/Lotnummer/
Číslo šarže/Αριθμός Παρτίδας/
Código do lote/Sarzzsám
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 produkcyj/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/
Fabbricante/Fabricant/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/Innehåller tillräckligt
till "96" test/Lze použit 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 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
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 rozmezí od do/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Hörmérséklettartomány/
Temperaturbegrænsning/
Przeznaczac 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/Sprawdz
w instrukcji obsługi/Dél 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

ORIG | MOU

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

ORIG | HUM

Human/Human/Humano/
Origine Humana/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

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.
- Reagents contain sodium azide (Na₃N) 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

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.
- Die Reagenzien enthalten Natriumazid (Na₃N) als Konservierungsmittel. Natriumazid kann mit Blei- und Kupferleitungen reagieren und hochexplosive Metallazide bilden. Spülen Sie die Leitungen beim Wegschütten 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) como 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 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
- 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 poiché 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 diagnostique in Vitro

- Pour usage professionnel seulement.
- Prière de se référer à la Publication N°: (CDC) 88-8395 de l'U.S. Département de 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.
- Réactifs contenant de l'Azide de Sodium (NaN_3) 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 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 laboratorisikkerhedsprocedurer eller andre lokale eller nationale forskrifter.
- Alle patientprøver skal behandles som potentielt smittefarlige.
- Reagenser indeholder natriumazid som præserveringsmiddel. Natriumazid kan danne eksplosive syrer i metalaflebs. Anvend korrekt affaldsprocedure.
- Følg lokale regler for afskaffelse af alt affald.

Advarsel

Alt materiale anvendt ved beregningen 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* διαγνωστική χρήση

- Για επαγγελματική χρήση, μόνο.
- Παρακαλούμαι όπως επικαλεστείτε τις οδηγίες ασφαλούς λειτουργίας των εργαστηρίων του Τμήματος Υγείας και Ανθρωπίνων 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 τόπου σχετικό Εθνικό κανονισμό.
- Μεταχειριστήτε όλα τα δείγματα ως μολυσμένα.
- Αποφύγετε επαφή με αντιδραστήρια που περιέχουν υπεροξειδίου 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

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). Εφόσον 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 laboratorisäkerhet.
- Hantera alla patientprover som potentiellt smittsamma.
- Vissa reagens innehåller natriumazid (NaN₃) 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.

CanAg CA19-9 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CA19-9 EIA kit is intended for the quantitative determination of the cancer associated antigen CA19-9 in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The CanAg CA19-9 EIA is based on a mouse monoclonal antibody, C192, highly specific for the sialyl Lewis^a epitope, also known as CA19-9 antigen (1). In adults, the epitope is typically expressed in trace amounts on mucosal cells of gastrointestinal epithelia. In patients with malignant disease, the epitope may appear associated with high molecular weight mucin in blood. Assays for CA19-9 are frequently used to monitor gastrointestinal malignancies such as pancreatic, gall bladder, gastric, and colorectal cancers (2, 3).

PRINCIPLE OF THE TEST

The CanAg CA19-9 EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, controls and patient samples are incubated together with a biotinylated Anti-CA19-9 monoclonal antibody (MAb) C192 (derived from mice) in streptavidin coated microstrips. CA19-9 present in calibrators or samples is adsorbed to the streptavidin coated microstrips by the biotinylated Anti-CA19-9 MAb during the incubation. The strips are then washed and incubated with HRP labeled Anti-CA19-9 MAb C192. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methyl-benzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color is proportional to the amount of CA19-9 present in the samples.

The color 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 CA19-9 concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA19-9 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

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

CA19-9 Calibrators	6 vials	2–8°C until expiry date stated on the vials
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CAL	CA19-9	0	0 U/mL	1 x 8 mL
CAL	CA19-9	15	15 U/mL	1 x 0.75 mL
CAL	CA19-9	30	30 U/mL	1 x 0.75 mL
CAL	CA19-9	60	60 U/mL	1 x 0.75 mL
CAL	CA19-9	120	120 U/mL	1 x 0.75 mL
CAL	CA19-9	240	240 U/mL	1 x 0.75 mL

CA19-9 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye and 0.05 % sodium azide as preservative. Ready for use.

CAL	CA19-9	0
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 should also be used for dilution of samples.

Component	Quantity	Storage and stability after first opening
CA19-9 Controls	2 vials	2–8°C until expiry date stated on the vials
CONTROL CA19-9 1	1 x 0.75 mL	
CONTROL CA19-9 2	1 x 0.75 mL	

CA19-9 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin and 0.05 % sodium azide as preservative. Ready for use.

BIOTIN	Anti-CA19-9
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Biotin Anti-CA19-9	1 x 15 mL	2–8°C until expiry date stated on the vial
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Biotin Anti-CA19-9 monoclonal antibody from mouse, approximately 2 µg/mL. Contains phosphate citrate-buffer (pH 7.5), bovine serum albumin, blocking agents, Tween 40, an inert red dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

CONJ	Anti-CA19-9
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Tracer, HRP Anti-CA19-9	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock Solution of HRP Anti-CA19-9 monoclonal antibody from mouse, approximately 40 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL	CONJ
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Tracer Diluent	1 x 15 mL	2–8°C until expiry date stated on the vial
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Phosphate citrate-buffer (pH 7.5) with bovine serum albumin, blocking agents, Tween 40, an inert blue dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
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SUBS	TMB
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TMB HRP-Substrate	1 x 12 mL	2–8°C until expiry date stated on the vial
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Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine (TMB). Ready for use.

STOP

Stop Solution	1 x 15 mL	2–8°C until expiry date stated on the vial
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Ready for use. Contains 0.12 M hydrochloric acid.

WASHBUF	25X
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Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
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To be diluted with water 25 times before use. A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative.

Indications of instability

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

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use

- 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.
- Avoid contact with reagents containing hydrogen peroxide or hydrochloric acid. In case of contact with any of these reagents, wash thoroughly with water.

- 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.

SPECIMEN COLLECTION AND HANDLING

The CanAg CA19-9 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. 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, 3 and 6 washing cycles, or semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid. The Nunc Immuno-8 manual strip washer is recommended if an automatic microplatewash is not used.
- 3. Microplate spectrophotometer**
With a wavelength of 620 nm and/or 405 nm, and an absorbance range of 0 to 3.0.

4. Precision pipettes

With disposable plastic tips for dispensing microlitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100 μ L is useful but not essential. Pipettes for dispensing millilitre volumes.

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 CA19-9 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 must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and patient specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. A careful washing procedure of the strips is essential. Ensure that each well is filled up completely to the top edge, that the aspiration of the wells between and after the washing cycles is complete and that the wells are empty. If there is moisture left, invert the plate and tap it carefully against absorbent paper.

Automatic strip washer: Follow the manufacturer's instructions for maintenance and wash the required number of wash cycles prior to and after each incubation step.

The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged giving poor liquid delivery and suction.

5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or respenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate solution.

Protocol Sheet

CanAg CA19-9 EIA REF 120-10

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

Step	Bottle/Plate	Procedure																																							
1. Prepare Wash Solution Prepare Tracer working solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionized water. Mix 50 µL of Tracer, HRP Anti-CA19-9 with 1 mL of Tracer Diluent per strip:																																							
	CONJ Anti-CA19-9 DIL CONJ																																								
		<table border="1"><thead><tr><th>No. of Strips</th><th>Tracer, HRP Anti-CA19-9 (µL)</th><th>Tracer Diluent (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><tr><td>9</td><td>450</td><td>9</td></tr><tr><td>10</td><td>500</td><td>10</td></tr><tr><td>11</td><td>550</td><td>11</td></tr><tr><td>12</td><td>600</td><td>12</td></tr></tbody></table>	No. of Strips	Tracer, HRP Anti-CA19-9 (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
No. of Strips	Tracer, HRP Anti-CA19-9 (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	<table border="1"> <tr> <td data-bbox="10 713 51 873">CAL</td> <td data-bbox="51 713 165 873">CA19-9</td> </tr> <tr> <td colspan="2" data-bbox="10 655 165 713">0, 15, 30, 60, 120, 240</td> </tr> <tr> <td data-bbox="10 655 51 873">CONTROL</td> <td data-bbox="51 655 165 873">CA19-9</td> </tr> </table>	CAL	CA19-9	0, 15, 30, 60, 120, 240		CONTROL	CA19-9	25 µL in each well
CAL	CA19-9							
0, 15, 30, 60, 120, 240								
CONTROL	CA19-9							
4. Add Biotin Anti-CA19-9	<table border="1"> <tr> <td data-bbox="176 713 227 873">BIOTIN</td> <td data-bbox="227 713 341 873">Anti-CA19-9</td> </tr> </table>	BIOTIN	Anti-CA19-9	100 µL in each well				
BIOTIN	Anti-CA19-9							
5. Incubate	<table border="1"> <tr> <td data-bbox="238 713 290 873">MICROPLA</td> </tr> </table>	MICROPLA	2 hour shaking at room temperature					
MICROPLA								
6. Wash	<table border="1"> <tr> <td data-bbox="300 713 352 873">MICROPLA</td> </tr> </table>	MICROPLA	Wash each well three times with Wash Solution. Use manual or automatic washer.					
MICROPLA								
7. Add Tracer working solution	<table border="1"> <tr> <td data-bbox="362 713 414 873">TRACER WORKING SOLUTION</td> </tr> </table>	TRACER WORKING SOLUTION	100 µL in each well					
TRACER WORKING SOLUTION								
8. Incubate	<table border="1"> <tr> <td data-bbox="424 713 476 873">MICROPLA</td> </tr> </table>	MICROPLA	1 hour shaking at room temperature					
MICROPLA								
9. Wash	<table border="1"> <tr> <td data-bbox="486 713 538 873">MICROPLA</td> </tr> </table>	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.					
MICROPLA								
10. Add TMB HRP-Substrate	<table border="1"> <tr> <td data-bbox="549 713 590 873">SUBS</td> <td data-bbox="590 713 600 873">TMB</td> </tr> </table>	SUBS	TMB	100 µL in each well				
SUBS	TMB							
11. Incubate	<table border="1"> <tr> <td data-bbox="611 713 663 873">MICROPLA</td> </tr> </table>	MICROPLA	30 min shaking at room temperature					
MICROPLA								
12. Read absorbance	<table border="1"> <tr> <td data-bbox="673 713 725 873">MICROPLA</td> </tr> </table>	MICROPLA	620 nm					
MICROPLA								
Alt.12 Add Stop Solution	<table border="1"> <tr> <td data-bbox="735 713 787 873">STOP</td> </tr> </table>	STOP	100 µL in each well					
STOP								
Alt.13 Incubate	<table border="1"> <tr> <td data-bbox="797 713 849 873">MICROPLA</td> </tr> </table>	MICROPLA	1 min shaking at room temperature					
MICROPLA								
Alt.14 Read absorbance	<table border="1"> <tr> <td data-bbox="859 713 911 873">MICROPLA</td> </tr> </table>	MICROPLA	Read at 405 nm within 15 min					
MICROPLA								

Preparation of reagents	Stability of prepared reagent
Wash Solution	2 weeks at 2–25°C in a sealed container
Tracer working solution	3 weeks at 2–8°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 deionised water to give a buffered Wash Solution.

Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-CA19-9 with 1 mL of Tracer Diluent per strip (see table below):

No. of Strips	Tracer, HRP Anti-CA19-9 (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 Tracer working solution.

Alternative: Pour the content of the Tracer, HRP Anti-CA19-9 into the vial of Tracer Diluent and mix gently. Make sure that the entire content of the Tracer, HRP Anti-CA19-9 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 both calibrators 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 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 CA19-9 Calibrators (CAL 0, 15, 30, 60, 120, 240), CA19-9 Controls (C 1, C 2) and patient specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 120	1st Unk				
B	Cal 0	Cal 120	1st Unk				
C	Cal 15	Cal 240	2nd Unk				
D	Cal 15	Cal 240	2nd Unk				
E	Cal 30	C1	etc.				
F	Cal 30	C1					
G	Cal 60	C2					
H	Cal 60	C2					

4. Add 100 µL of Biotin Anti-CA19-9 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 surface of the liquid.
5. Incubate the plate for 2 hours (± 5 min) at room temperature (20-25°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 μ L of Tracer working solution to each well. Use the same pipetting procedure as in item 4 above.
8. Incubate the frame for 1 hour (\pm 5 min) at room temperature (20–25°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 μ L of TMB HRP-Substrate to each well using the same pipetting technique as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between addition to the first and last well should not exceed 5 min.
11. Incubate for 30 min (\pm 5 min) at room temperature with constant shaking. Avoid exposure to 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 reader capable of reading at 620 nm, the absorbance can be determined as in item alternative 12:

- Alt. 12. Add 100 μ L of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg CA19-9 EIA measures concentrations between 1 and 240 U/mL. If CA19-9 concentrations above the measuring range are to be expected, it is recommended to dilute samples with CA19-9 Calibrator 0 prior to analysis.

Quality control

CA19-9 Control 1 and 2 should 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 materials

Since no common reference material is available for CA19-9 antigen, CanAg CA19-9 EIA Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer with built-in data calculation program is used, refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the CA19-9 calibrators.

For automatic calculation of CA19-9 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 evaluation methods should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CA19-9 calibrator against the corresponding CA19-9 concentration (in U/mL), see figure below. The unknown CA19-9 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 CA19-9 levels higher than 240 U/mL the samples should be diluted 1/10 and 1/100 with CA19-9 Calibrator 0 to obtain the accurate CA19-9 concentration of the samples.

1/10 dilution = 50 μ L of specimen + 450 μ L of CA19-9 Calibrator 0

1/100 dilution = 50 μ L of 1/10 dilution + 450 μ L of CA19-9 Calibrator 0

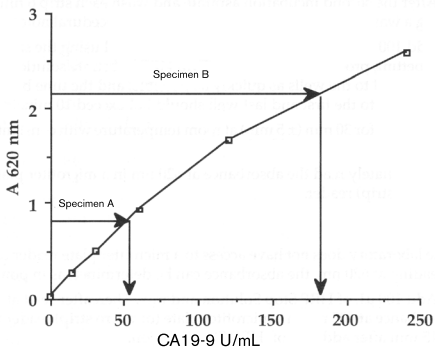
The CA19-9 concentration of the undiluted sample is then calculated as:

Dilution 1/10: 10 x measured value

Dilution 1/100: 100 x measured value

Example of results

Specimen			Calibrator values	Mean abs value (A)	CA19-9 U/mL
CAL	CA19-9	0	0 U/mL	0.036	
CAL	CA19-9	15	15 U/mL	0.276	
CAL	CA19-9	30	30 U/mL	0.508	
CAL	CA19-9	60	60 U/mL	0.928	
CAL	CA19-9	120	120 U/mL	1.665	
CAL	CA19-9	240	240 U/mL	2.583	
Specimen A				0.815	52
Specimen B				2.130	181



Example, do not use this curve to determine assay results.

LIMITATIONS OF THE PROCEDURE

The level of CA19-9 cannot be used as absolute evidence for the presence or absence of malignant disease and the CA19-9 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 CA19-9 test should not replace any established clinical examination.

Benign conditions such as acute or chronic pancreatitis or cholelithiasis may cause elevated levels of CA19-9. Patients with a Le^{a-/b-} phenotype do not express the CA19-9 reactive epitope. 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

CA19-9 was measured in 100 healthy blood donors, 36 women and 64 men. The mean value obtained was 6.5 U/mL with a standard deviation of 6.4. The median value was 4.5 U/mL, range 0 – 29 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=100

Fraction	Reference limit (U/mL)
2.5 th (lower)	0
97.5 th (upper)	25

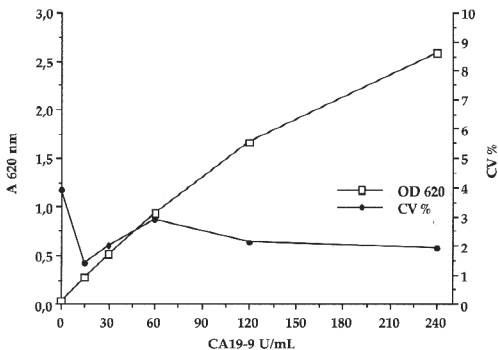
100% of the healthy subjects had assay values below 37 U/mL.

It is recommended that each laboratory establish its own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc.

PERFORMANCE CHARACTERISTICS

Dose-response and precision profile

A typical calibration curve and precision profile obtained with the CanAg CA19-9 EIA kit are shown below. The precision profile is based on random pipetting of calibrators into one microplate, n = 10.



Precision

Total precision was determined according to NCCLS guideline EP5-A (4) using four levels of frozen pooled human serum containing added ascitespool. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days. The analyses were undertaken during a period of 53 months, by \geq three different technicians and using 20 different CanAg CA19-9 EIA kit batches.

Sample	Replicates	Mean U/mL	Within-run SD (U/mL)	Within-run CV %	Between-day SD (U/mL)	Between-day CV %
CA19-9 1	80	15.4	0.6	3.8	1.0	6.8
CA19-9 2	80	56.3	1.9	3.3	3.6	6.3
CA19-9 3	80	99.8	4.5	4.5	6.2	6.2
CA19-9 4	80	182	7.9	4.4	12	7.0

Detection limit

The detection limit of the CanAg CA19-9 EIA assay is < 1 U/mL defined as the concentration corresponding to the mean of the absorbance values for the CA19-9 Calibrator 0 plus 2 standard deviations according to the formula:

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

Recovery

Spiked serum samples were prepared by adding different levels of human CA19-9 antigen to normal serum samples. The recovery of the antigen was in the range 90–110%.

Hook effect

No hook effect has been noticed with samples up to 50 000 U/mL. **NOTE:** In very high samples the color 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

Eight patient samples were serially diluted with CA19-9 Calibrator 0 and analyzed. The obtained values were between 96–105 % of the expected values in the range 10–200 U/mL.

Specificity

The monoclonal antibody used (C192) is highly specific for the sialyl Lewis^a epitope (1). The NCCLS guideline EP7-P (5) 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	0.6 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.

LITERATURE REFERENCES

1. Rye P. D. et al., (1998) Summary report on the ISOBM TD-6 workshop: Analysis of 20 monoclonal antibodies against Sialyl Lewis^a and related antigens. Montreaux, Switzerland; September 19-24 1997. *Tumor Biol.* 19: 390-420.
2. Eskelinen M., and Haglund U. (1999) Developments in serologic detection of human pancreatic adenocarcinoma. *Scand J Gastroenterol.* 34: 833-844.
3. Hammarström S., and Stigbrand T. (2002) Gastric Cancer. In "Tumor markers, Physiology, Pathobiology, Technology and Clinical Applications" Eds. Diamandis E. P. et al., AACCC Press, Washington pp 375-379.
4. National Committee for Clinical Laboratory Standards, Evaluation of Precision Performance of Clinical Chemistry Devices. Approved Guideline EP5-A (1999).
5. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation protocol Number 7, Vol. 6, No 13, August (1986).



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