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

CanAg AFP EIA

REF 600-10

IVD

CE

Instructions for use. 2010-04

EN	**EXPLANATION OF SYMBOLS**
BG	**ОБЯСНЕНИЕ НА СИМВОЛИТЕ**
CS	**VÝZNAM SYMBOLŮ**
DA	**SYMBOLFORKLARING**
DE	**ERKLÄRUNG DER SYMBOLE**
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	**SIGNIFICADO DE LOS SÍMBOLOS**
ET	**SÜMBOLITE SELGITUS**
FR	**EXPLICATION DES SYMBOLES**
HR	**OBJAŠNJENJE SIMBOLA**
HU	**JELMAGYARÁZAT**
IT	**SPIEGAZIONE DEI SIMBOLI**
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	**SIMBOLU SKAIDROJUMS**
NL	**VERKLARING DER SYMBOLEN**
NO	**SYMBOLFORKLARING**
PL	OBJAŚNIENIE SYMBOLI
PT	**EXPLICAÇÃO DOS SÍMBOLOS**
RO	SEMNIFICAŢIA SIMBOLURILOR
RU	ОБОНАЧЕНИЯ
SE	**SYMBOLFÖRKLARING**
SK	**VÝZNAM SYMBOLOV**
SL	**RAZLAGA SIMBOLOV**
SR	**OBJAŠNJENJE SIMBOLA**
TR	SEMBOLLERİN AÇIKLAMALARI

Use By/Годно до/Použitelné do/ Holdbar til/Verwendbar bis/ Ημερομηνία λήξης/Fecha de caducidad/Kõlblik kuni/ Utiliser jusque/Rok valjanosti/ Felhasználható/Utilizare entro/ Sunaudoti iki/Izlietot līdz/Houdbaar tot/Brukes innen/Użyć przed/ Prazo de validade/Expiră la/ Использовать до/Använd före/ Použite´né do/ Uporabno do/ Upotrebljivo do/Son Kullanma Tarihi

LOT

Batch code/Номер на партида/ Číslo šarže/Lotnummer/ Chargenbezeichnung/Αριθμός Παρτίδας/Código de lote/Partii kood/Code du lot/Kod serije/ Sarzsszám/Codice del lotto/ Partijos kodas/Partijas kods/Lot nummer/Partikode/Kod partii/ Código do lote/Număr de lot/ Номер лота/Lotnummer/Číslo šarže/Številka serije/Kod partije/ Parti Kodu

Date of manufacture/Дата на производство/Datum výroby/ Produktionsdato/Herstellungsdatum/ Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/ Date de fabrication/Datum proizvodnje/ Gyártási idő/Data di produzione/ Pagaminimo data/Ražošanas datums/ Productiedatum/Fremstillingsdato/ Data produkcji/Data de fabrico/Data fabricației/Дата производства/ Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

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

IVD

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

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilstrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιεχόμενο επαρκές για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour “96” tests/Sadrži dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per “96” saggi/Turinys skirtas atlikti <96> tyrimus/ Saturs pietiekams <96> testiem/Inhoud voldoende voor “96” testen/til “96” test/ Tilstrekkelig innhold for <96> prøver/ Wystarczy na wykonanie <96> testów/ Conteúdo suficiente para “96” ensaios/ Conținut suficient pentru 96 de teste/ Съдържит достаточные количества для «96» определений/Innehåller tillräckligt till “96” antal tester/Obsah postačuje na tento počet testov: <96>/Vsebina zadostuje za <96> testov/Sadržina dovoljna za <96> testova/<96> testleri için yeterlilik içerir

REF

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

Consult Instructions for Use/ Прочетете инструкцията за употреба/Konzultujte s návodem k použití/De brugsanvisning/Siehe Gebrauchsanweisung/Συμβουλευτείτε τις Οδηγίες σχετικά με τη χρήση/ Consulte las instrucciones de uso/ Vt kasutusjuhendit/Consulter le mode d'emploi/Pročitajte upute za uporabu/ Olvassa el a használati utasítást/ Consultare le istruzioni per l'uso/Dėl naudojimo žiūrėkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene før bruk/Sprawdzić w instrukcji użycia/ Consulte as Instruções de Utilização/ Consultaţi instrucţiunile de utilizare/ Обратитесь к инструкции по применению/Se bruksanvisning/ Prečítajte si návod na používanie/ Pročitajte uputstvo za upotrebu/ Kullanım Talimatlarına Bakınız

CONT

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

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

ORIG | HUM

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

ORIG | MOU

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

ORIG | BOV

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

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

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

WARNINGS AND PRECAUTIONS

EN

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.

ADVARSLER OG FORHOLDSREGLER

DA

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.

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.

ΠΡΟΕΙΔΟΠΟΙΗΣΕΙΣ ΚΑΙ ΠΡΟΦΥΛΑΞΕΙΣ

EL

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 Ηπατίδας Β Αντι**γόνο 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.

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.

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.

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.

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 AFP EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg AFP EIA kit is intended for the quantitative determination of α-Fetoprotein (AFP) in human serum.

SUMMARY AND EXPLANATION OF THE ASSAY

α-Fetoprotein (AFP), the foetal equivalent to albumin, is a 67 kDa glycoprotein produced during embryonic development and found in high concentrations in foetal serum and amniotic fluid. In normal non-pregnant adults AFP is present in low concentrations in serum. However AFP may be markedly increased in the serum from patients with cancer of the liver, testis or ovary. Quantitative determination of AFP in serum may be valuable in the management of patients with suspected or diagnosed liver cancer or germ cell tumours of the testis or ovary (1, 2).

PRINCIPLE OF THE TEST

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

REAGENTS

- Each CanAg AFP 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.

AFP Calibrators			6 vials	2–8°C until expiry date stated on the vials
CAL	AFP	0	0 µg/L	1 x 0.75 mL
CAL	AFP	5	5 µg/L	1 x 0.75 mL
CAL	AFP	25	25 µg/L	1 x 0.75 mL
CAL	AFP	100	100 µg/L	1 x 0.75 mL
CAL	AFP	250	250 µg/L	1 x 0.75 mL
CAL	AFP	500	500 µg/L	1 x 0.75 mL

Human AFP 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
AFP Controls	2 vials	2–8°C until expiry date stated on the vials
CONTROL AFP 1	1 x 0.75 mL	
CONTROL AFP 2	1 x 0.75 mL	

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

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

Biotin Anti-AFP monoclonal antibody from mouse, approximately 1 µg/mL. Contains phosphate buffered saline (pH 7.2), bovine serum albumin, bovine immunoglobulin, blocking agents, Tween 20, an inert blue dye and 0.01% methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-AFP before use.

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

Stock solution of HRP Anti-AFP monoclonal antibody from mouse, approximately 20 µg/mL. Contains preservatives. To be mixed with Biotin Anti-AFP before use.

SUBS TMB		
TMB HRP-Substrate	1 x 12 mL	2–8°C until expiry date stated on the vial

Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

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

Indications of instability

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

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SPECIMEN COLLECTION AND HANDLING

The CanAg AFP 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 2 days. 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 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.

Protocol Sheet

CanAg AFP EIA REF 600-10

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-AFP BIOTIN Anti-AFP	Mix 50 µL of Tracer, HRP Anti-AFP, with 1 mL of Biotin Anti-AFP per strip:

No. of Strips	Tracer, HRP Anti-AFP (µL)	Biotin Anti-AFP (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10

		11	550	11
		12	600	12
2. Wash	MICROPLA	Wash each well once with wash solution		
3. Add calibrators, controls and samples	CAL AFP 0, 5, 25, 100, 250, 500 CONTROL AFP 1, 2	25 µL in each well		
4. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well		
5. Incubate	MICROPLA	1 hour shaking at room temperature		
6. Wash	MICROPLA	Wash each well six times with wash solution		
7. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well		
8. Incubate	MICROPLA	30 min shaking at room temperature		
9. Read absorbance	MICROPLA	620 nm		
Alt.9 Add Stop Solution	STOP	100 µL in each well		
Alt.10 Incubate	MICROPLA	1 min shaking at room temperature		
Alt.11 Read absorbance	MICROPLA	Read at 405 nm within 15 min		

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg AFP EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25°C) prior to use. The assay should only be performed at temperatures between 20–25°C to obtain accurate results. Frozen specimens should be brought to room temperature slowly and must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators, controls and patient specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.
   - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. It's highly recommended to use *strip* process mode and *overflow* wash mode with a dispensing volume of 800 µL. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or respenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate Solution.

Preparation of reagents	Stability of prepared reagent
Wash Solution	2 weeks at 2–25°C in a sealed container

Pour the 50 mL Wash Concentrate into a clean container and dilute 25- fold by adding 1200 mL of distilled or deionized water to give a buffered Wash Solution.

Antibody Solution	3 weeks at 2–8°C

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

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

NOTE: The Antibody Solution is stable for 3 weeks at 2–8°C. Do not prepare more Antibody Solution than will be used within this period and make sure that it is stored properly.

Assay procedure

Perform each determination in duplicate for calibrators, controls and patient samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25°C) before use.

1. Start to prepare Wash Solution and Antibody Solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 25 µL of the AFP Calibrators (CAL 0, 5, 25, 100, 250, 500), 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 250	Unk 1				
B	Cal 0	Cal 250	Unk 1				
C	Cal 5	Cal 500	Unk 2				
D	Cal 5	Cal 500	Unk 2				
E	Cal 25	C1	etc.				
F	Cal 25	C1					
G	Cal 100	C2					
H	Cal 100	C2					

4. Add 100 µL of Antibody Solution to each well using a 100 µL precision pipette (or an 8-channel 100 µL precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.
5. Incubate the frame containing the strips for 1 hour (± 5 min) at room temperature (20–25°C) with constant shaking of the plate using a microplate shaker.
6. Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.

7. Add 100 µL of TMB HRP-Substrate to each well using the same pipetting procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the addition to the first and last well should not exceed 5 min.
8. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.
9. 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. 9. 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 AFP EIA measures concentrations between 0.5 and 500 µg/L. If AFP 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 should also be measured in order to determine the endogenous AFP concentration (see "Calculation of results").

Quality control

AFP 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 accuracy of the assay.

Reference material

The 1st International Standard IS 72/225 may be used as a reference standard. Values for AFP Calibrators and Controls were assigned against a set of in-house reference standards whose values are traceable to IS 72/225 using the conversion factor 0.83, i.e. 1µg/L corresponds to 0.83 kIU/L.

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

- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/L.

NOTE: 4-parametric or linear regression should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each AFP calibrator against the corresponding AFP concentration (in µg/L), see figure below. The unknown AFP 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 AFP levels higher than 500 µg/L the samples should be diluted 1/10 and 1/100 with normal human serum and reanalyzed to obtain the accurate AFP concentration. **NOTE**: The sample used for dilution should also be measured in order to determine the endogenous AFP concentration.

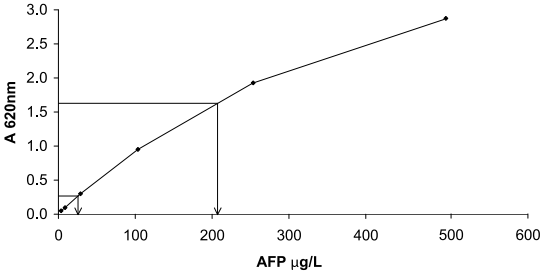
The AFP concentration of the undiluted sample is then obtained as follows:

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

Dilution 1/100: $100 \times ([AFP]_{Diluted\ sample} - (0.99 \times [AFP]_{Normal\ serum}))$

Example of results

Specimen	Calibrator values	Mean abs value (A)	AFP (µg/L)
CAL AFP 0	0 µg/L	0.036	
CAL AFP 5	5 µg/L	0.083	
CAL AFP 25	25 µg/L	0.282	
CAL AFP 100	100 µg/L	0.938	
CAL AFP 250	250 µg/L	1.914	
CAL AFP 500	500 µg/L	2.854	
Specimen A		0.220	19.4
Specimen B		1.686	208



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

LIMITATIONS OF THE PROCEDURE

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

AFP was measured in 40 apparently healthy men and 93 apparently healthy women. The mean value obtained was 2.8 µg/L with a standard deviation of 2.6. 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 (µg/L)	90% confidence interval
2.5th (lower)	0.1	0.0–0.3
97.5th (upper)	10	8.7–14.6

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. As AFP levels have been shown to increase with age, the use of age-specific reference intervals have been suggested (2, 3). 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 (3, 4).

PERFORMANCE CHARACTERISTICS

Precision

Total precision was calculated according to NCCLS guideline EP5-A (6) using four levels of frozen pooled human serum containing added AFP and nine different CanAg AFP EIA reagent combinations. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days.

Sample	Replicates	Mean (µg/L)	Within-run SD (µg/L)	Within-run CV %	Between-day SD (µg/L)	Between-day CV %
AFP 1	80	7.8	0.2	2.0	0.1	1.8
AFP 2	80	23.2	0.4	1.8	0.3	1.4
AFP 3	80	207	3.5	1.7	3.5	1.7
AFP 4	80	416	6.6	1.6	8.5	2.0

Detection limit

The detection limit of the CanAg AFP EIA is $\leq$ 0.5 µg/L defined as the concentration corresponding to the mean of the absorbance values of the AFP calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 5 - OD\ CAL\ 0} \times 5\ \mu g/L$$

Recovery

Spiked serum samples were prepared by adding aliquots of a sample with highly elevated AFP to normal serum samples. The recovery of the added antigen was in the range 90–106 %. **NOTE:** Recovery studies should **not** be performed using the kit calibrators.

Hook effect

No hook effect has been noticed for samples up to 40 000 µg/L. However, since patients with advanced hepatocellular carcinoma may show extremely high levels, false low results due to a high dose hook effect may be seen in specimens from these patients. In order to avoid reporting misleadingly low results due to a hook effect at higher concentrations, particularly in patients for whom markers are being measured for the first time, or when very high AFP values may be expected, it is recommended to assay specimens at two dilutions (i.e. neat and diluted 1:100 with normal human serum).

Linearity

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

Specificity

The CanAg AFP EIA is based on two mouse monoclonal antibodies, AFPK51 and AFPK57, targeting two separate antigenic determinants on the AFP molecule (5). The NCCLS guideline EP7-P (7) 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	2 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.

REFERENCES

1. Johnson, P.J, (2002) Tumor Markers in Primary Malignancies of the liver. In "Tumor Markers: Physiology, pathobiology, technology and clinical applications", ed. Dimandis E.P. AACC Press, Washingon pp 269-276.
2. Stenman, U-H and Alfthan, H. (2002) Markers for Testicular Cancer.In "Tumor Markers: Physiology, pathobiology, technology and clinical applications", ed. Dimandis E.P. AACC Press, Washingon, pp 351-359.
3. Christiansen, M., Hogdall, C.K., Andersen, J.R. and Norgaard-Pedersen, B. (2001) Alpha-fetoprotein in plasma and serum of healthy adults: preanalytical, analytical and biological sources of variation and construction of age-dependent reference intervals. *Scand J Invest* 61: 205-216.
4. Trapé, J., Botargues, J.M., Porta, F., Ricós, C., Badal, J.M., Salinas, R., Sala, M., and Roca, A. (2003) Reference change value for α-Fetoprotein and its application in early detection of hepatocellular carcinoma in patients with hepatic disease. *Clin Chem* 49(7): 1209-1211.
5. Nustad, K., Paus, E., Kierulf, B., and Bormer, O.P. (1998) Specificity and affinity of 30 monoclonal antibodies against Alpha-Fetoprotein. *Tumor Biol* 19: 293-300.
6. National Committee for Clinical Laboratory Standards, Evaluation of Precision Performance of Clinical Chemistry Devices. Approved Guideline EP5-A (1999).
7. 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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