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

CYFRA 21-1 EIA

IVD

REF 211-10

CE

Instructions for use. 2011-05

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	SĪMBOLU 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

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

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 fabricaţiei/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/Diagnostický zdravotnický prostředek 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 diagnostice 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/Conţinut suficient pentru 96 de teste/Содержит 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/Catalogusnummer/Katalognummer/Numer katalogowy/Número do catálogo/Număr de catalog/Номер по каталогу/Produktnummer/Katalógové číslo/Kataloška številka/Kataloški broj/Katalog numarası

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Consult Instructions for Use/ Прочетете инструкцията за употреба/Konzultujte s návodem k použití/Se 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 soupravy/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/ Biološki 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/ Biološki rizici/Biyolojik riskler

ORIG **HUM**

Human/С човешки произход/Lidské/ Humant/Human/δείγματα αναφοράς/ Humano/Inimäritolu/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/Maus/απο 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/Mišjeg 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/Atkurti, ištirpdant su/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

Distributed by MyBioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

EN

CYFRA 21-1 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CYFRA 21-1 EIA kit is intended for the determination of soluble cytokeratin 19 fragments in human serum. The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.

SUMMARY AND EXPLANATION OF THE ASSAY

Cytokeratin 19 is a member of a family of at least twenty different cytokeratin polypeptides. Cytokeratins form the intermediate filament structure of epithelial cells (1, 2). Cytokeratin filaments are poorly soluble but following proteolytic degradation, soluble cytokeratin fragments are formed and released into body fluids.

CYFRA 21-1 is an immunoassay that determines the level of cytokeratin 19 fragments in serum (3-6). The CYFRA 21-1 EIA is based on two monoclonal antibodies (BM 19.21 and KS 19.1) specific for cytokeratin 19 (3, 7-8). Elevated levels of cytokeratin 19 fragments are seen in serum from patients with lung cancer (5, 9-12) and also in other cancers e. g. bladder cancer (13). In patients with lung cancer, CYFRA 21-1 has been reported to be useful for monitoring the course of disease during treatment and for detection of recurrence (11, 14-17).

PRINCIPLE OF THE TEST

The CYFRA 21-1 EIA is a solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of soluble fragments of cytokeratin 19 (7-8). Calibrators, controls and patient samples are incubated together with biotinylated Anti-CYFRA 21-1 MAb and horseradish peroxidase (HRP) labeled Anti-CYFRA 21-1 MAb in streptavidin coated microstrips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) 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 development is proportional to the amount of CYFRA 21-1 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).

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CYFRA 21-1 concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CYFRA 21-1 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 use
MICROPLA		
Microplate	1 Plate	2–8°C until expiry date stated on the plate
12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminum pouch, containing desiccant. Reseal carefully to keep dry.		
CAL | CYFRA 21-1 | A		
CYFRA 21-1 Calibrator A	1 x 8 mL	2–8°C until expiry date stated on the vial
Phosphate buffered salt solution containing bovine serum albumin, an inert yellow dye, and a non-azide antimicrobial preservative. Ready for use. Should also be used for dilution of samples.		

Distributed by MyBioSource.com

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Component	Quantity	Storage and stability after first use
CYFRA 21-1 Calibrators B-F	5 vials, lyophilized	Stability after reconstitution 4 weeks at 2–8°C 4 months at -20°C or below
CAL CYFRA 21-1 B	1 x 1 mL	
CAL CYFRA 21-1 C	1 x 1 mL	
CAL CYFRA 21-1 D	1 x 1 mL	
CAL CYFRA 21-1 E	1 x 1 mL	
CAL CYFRA 21-1 F	1 x 1 mL	

The lyophilized calibrators contain CYFRA 21-1 antigen in a phosphate buffered salt solution containing bovine serum albumin, an inert yellow dye, and a non-azide antimicrobial preservative. To be reconstituted with distilled or deionized water before use. **NOTE:** The exact CYFRA 21-1 concentration is lot specific and is indicated on the label of each vial.

CYFRA Controls	2 vials, lyophilized	Stability after reconstitution 1 week at 2–8°C 4 months at -20°C or below
CONTROL CYFRA 21-1 1	1 x 1 mL	
CONTROL CYFRA 21-1 2	1 x 1 mL	

The lyophilized controls contain CYFRA 21-1 antigen in a human serum matrix and a non-azide antimicrobial preservative. To be reconstituted with distilled or deionized water before use.

Distributed by MyBioSource.com

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Component	Quantity	Storage and stability after first use
BIOTIN Anti-CYFRA 21-1		
Biotin Anti-CYFRA 21-1	1 x 15 mL	2–8°C until expiry date stated on the vial

Biotin Anti-CYFRA 21-1 monoclonal antibody from mouse, approximately 1.25 µg/mL. Contains Tris-HCl buffered salt solution (pH 7.2), bovine serum albumin, blocking agents, detergent, an inert blue dye, and a non-azide antimicrobial preservative. To be mixed with Tracer before use.

CONJ Anti-CYFRA 21-1		
Tracer, HRP Anti-CYFRA 21-1	1 x 0.75 mL	2–8°C until expiry date stated on the vial

Stock Solution of HRP Anti-CYFRA 21-1 monoclonal antibody from mouse, approximately 42 µg/mL. Contains non-azide antimicrobial preservatives. To be mixed with Biotin Anti-CYFRA 21-1 prior to 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' tetra-methylbenzidine (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.

Distributed by MyBioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Component	Quantity	Storage and stability after first 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 distilled or deionized water 25 times before use.

Indications of instability

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

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use:

- Follow the instructions in the package insert. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Handle all patient specimens as potentially infectious. It is recommended that human source reagent and human specimens are handled in accordance with the OSHA Standard on Bloodborne pathogens (19). Biosafety level 2 (20) or other appropriate biosafety practices should be used for material that contain or are suspected of containing infectious agents.
- Avoid contact with reagents containing hydrogen peroxide or hydrochloric acid. In case of contact with any of these reagents, wash thoroughly with water.
- Follow local guidelines for disposal of all waste material.

Caution

Each donor unit 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.

Distributed by MyBioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

SPECIMEN COLLECTION AND HANDLING

The CYFRA 21-1 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 1 day. For longer periods store samples at -40°C or below. Avoid repeated freezing and thawing of the samples. If aliquoted choose the right sized tube i.e. limit the empty space in the tube. Bring frozen samples to room temperature and mix thoroughly by gentle inversion before analysis. *Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.*

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous, approximately 900-1100 oscillations/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 microplate washer 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 microliter volumes. An 8-channel pipette or dispenser pipette with disposable plastic tips for delivery of 100 µL is recommended but not required. Pipettes for dispensing milliliter volumes.
5. **Distilled or deionized water**
   For reconstitution of CYFRA 21-1 Calibrators, CYFRA 21-1 Controls and for preparation of diluted wash solution.

tributed by MyBioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CYFRA 21-1 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. Frozen specimens must be gently but thoroughly mixed by gentle inversion after thawing. *Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.* If aliquoted choose the right sized tube i.e. limit the empty space in the tube.
3. Before starting to pipette calibrators and unknown 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 to contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial into 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 dispenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper precision pipetting technique when handling samples and reagents. Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over. A diligent pipetting technique is of particular importance when handling the samples and the TMB HRP-Substrate solution.

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Preparation of reagents	Stability of prepared reagent
CYFRA 21-1 Calibrators	4 weeks at 2–8°C 4 months at -20°C or below

Add exactly 1.0 mL of distilled water to each vial. Allow to stand for at least 15 minutes to reconstitute and mix gently before use. **NOTE:** The concentration of the calibrators is stated on the labels and should be used for calculation of the results. Mix only by gentle swirling or inversion. *Mixing of calibrators using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.*

CYFRA 21-1 Controls	1 week at 2–8°C 4 months at -20°C or below

Add exactly 1.0 mL of distilled water to each vial. Allow to stand for at least 15 minutes to reconstitute and mix gently before use. **NOTE:** The range of the controls is stated on the labels. Mix only by gentle swirling or inversion. *Mixing of controls using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.*

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	1 day at 2–8 °C

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-CYFRA 21-1 with 1 mL of Biotin Anti-CYFRA 21-1 per strip (see table below):

tributed by MyBioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

No. of Strips	Tracer, HRP Anti-CYFRA 21-1 (µL)	Biotin Anti-CYFRA 21-1 (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 tube for preparation of Anitbody Solution.

ASSAY PROCEDURE

Perform each determination in duplicate for calibrators, controls and unknown 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 CYFRA 21-1 Calibrators, Controls 1 & 2, 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 aluminum 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. Mix samples by gentle inversion. *Do not Vortex for more than 1 second.* Pipette 50 µL of the CYFRA 21-1 Calibrators (CAL A, B, C, D, E, and F), Controls 1 & 2 and unknown specimens (unknowns-Unk) into the strip wells according to the following scheme:

Distributed by IVD BioSource.com

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	1st Unk				
B	Cal A	Cal E	1st Unk				
C	Cal B	Cal F	2nd Unk				
D	Cal B	Cal F	2nd Unk				
E	Cal C	C1					
F	Cal C	C1					
G	Cal D	C2					
H	Cal D	C2					

4. Add 100 µL of Antibody Solution to each well using a 100 µL 8-channel precision pipette (or a 100 µL precision pipette). Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over.
5. Incubate the plate for 1 hour (± 5 min) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker.
6. After the incubation aspirate and wash each strip 6 times.
7. Add 100 µL of TMB HRP-Substrate to each well using the same procedure 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.
8. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid exposure to 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 in the alternative item 9 below:

Alt. 9. Add 100 µL of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Distributed By BioSource

SEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCE

Distributed by MVB Source

Protocol Sheet

CYFRA 21-1 EIA REF 211-10

Prepare the components directly before use. Use wash and incubation conditions according to the Instructions.

Step		Vial/Plate	Procedure
1.	Prepare CYFRA 21-1 Calibrators	CAL | CYFRA 21-1 B, C, D, E, F	Add 1 mL of distilled water to each vial. Allow to stand for at least 15 minutes and mix gently. NOTE: The exact concentration of each calibrator/ controls is stated on the label. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.
	Prepare CYFRA 21-1 Controls	CONTROL | CYFRA 21-1 1, 2	
	Prepare Wash Solution	WASHBUF | 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionized water.
	Prepare Antibody Solution	CONJ | Anti-CYFRA 21-1 BIOTIN | Anti-CYFRA 21-1	Mix 50 µL of Tracer, HRP Anti-CYFRA 21-1 with 1mL of Biotin Anti-CYFRA 21-1 per strip:

No. of Strips	Tracer, HRP Anti-CYFRA 21-1 (µL)	Biotin Anti-CYFRA 21-1 (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

		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 | CYFRA 21-1 A, B, C, D, E, F CONTROL | CYFRA 21-1 1, 2	50 µL in each well. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second.
4.	Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well
5.	Incubate	MICROPLA	1 hour shaking at 20-25°C
6.	Wash	MICROPLA	Wash each well six times with Wash Solution Use manual or automatic washer.
7.	Add TMB HRP-Substrate	SUBS | TMB	100 µL in each well
8.	Incubate	MICROPLA	30 min shaking at 20-25°C
9.	Read absorbance	MICROPLA	620 nm
Alt.9	Add Stop Solution	STOP	100 µL in each well
Alt.10	Mix	MICROPLA	Allow to mix at 20-25°C
Alt.11	Read absorbance	MICROPLA	Read at 405 nm within 15 min

Measurement range

The CYFRA 21-1 EIA measures concentrations between 0.5 and approximately 50 ng/mL. If CYFRA 21-1 concentrations above the measuring range are expected, it is recommended to dilute samples with CYFRA 21-1 Calibrator A prior to analysis (see "Calculation of results with diluted samples").

Quality control

CYFRA 21-1 Control 1 and 2 should be used for validation of each assay series. Ranges of expected results are indicated on the vial labels.
The CYFRA 21-1 assay results should be considered valid if:

- The mean values of control duplicates are within the specified ranges.
- The duplicate replicates of calibrators B-F and controls do not exceed a CV of 15% based on the absorbance readings.
- The duplicate replicates of controls do not exceed a CV of 15.0% based on the concentration values.
- The duplicate replicates of calibrator A (zero) are not more than 0.06 OD units different from each other.

If an assay results in invalid calibrator or control results, a complete check of reagents, accuracy of pipettes, plate washer and reader performance should be made and the analysis repeated. Each laboratory may also prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

Reference material

Since no common reference material is available for CYFRA 21-1 antigen, CYFRA 21-1 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 CYFRA 21-1 calibrators.

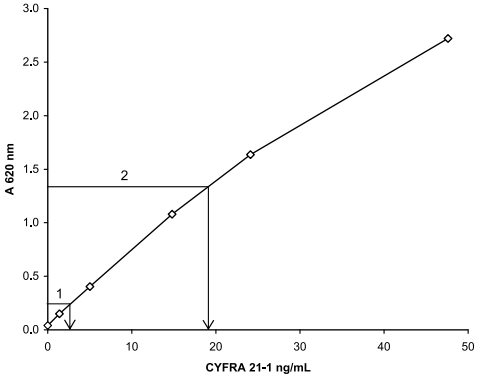
For automatic calculation of CYFRA 21-1 results, it is recommended to use the cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 ng/mL.

CYFRA 21-1 results shown in this package insert, were calculated using the cubic spline curve fit from Molecular Devices SOFTmax® PRO software. This curve fit generates a fit to a cubic equation between each pair of data points. The general form of a cubic equation is: $y=A+Bx+Cx^2+Dx^3$.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CYFRA 21-1 calibrator against the corresponding CYFRA 21-1 concentration (in ng/mL). The unknown CYFRA 21-1 concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

Example of results

Specimen	Calibrator values (ng/mL)	Mean abs value (A)	CYFRA 21-1 ng/mL
Calibrator A	0	0.041	
Calibrator B	1.4	0.151	
Calibrator C	5.0	0.405	
Calibrator D	14.8	1.080	
Calibrator E	24.1	1.635	
Calibrator F	47.6	2.721	
Specimen 1		0.259	2.9
Specimen 2		1.366	19.4



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

The exact CYFRA 21-1 concentration is indicated on the label of each calibrator vial.

Note: The evaluation method of calculating CYFRA 21-1 results should be used consistently when used in the course of monitoring a patient.

Calculation of results with diluted samples

Samples with CYFRA 21-1 concentrations above the measuring range can be diluted with CYFRA 21-1 Calibrator A. The recommended dilution is 1/2.

- 1/2 dilution = 100 µL of specimen + 100 µL of CYFRA 21-1 Calibrator A
  The CYFRA 21-1 concentration of the diluted sample is then calculated as:
- Dilution 1/2 : 2 x measured value

LIMITATIONS OF THE PROCEDURE

Patients with confirmed lung cancer may have CYFRA 21-1 values in the same range as healthy subjects. Elevated levels of CYFRA 21-1 may also be found in subjects with non-malignant disease e.g. renal failure and certain benign respiratory diseases (13, 18). Therefore, the level of CYFRA 21-1 cannot be used as absolute evidence for the presence or absence of malignant disease and the CYFRA 21-1 EIA 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 CYFRA 21-1 test should not replace any established clinical examination. The performance of the assay has not been adequately validated in small cell lung cancer (SCLC), large cell carcinoma and Stage I and II lung cancers.

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. *Excessive mixing may result in artifactually decreased CYFRA 21-1 values, therefore mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 1 second. If samples are aliquoted, limit the empty space of the tube to avoid stress during mixing.*

EXPECTED VALUES

The distribution of CYFRA 21-1 levels determined in 875 specimens is shown in the table below:

Distribution of CYFRA 21-1 Assay Values

	Number of subjects	0 – 1.8 ng/mL	1.9 – 5.0 ng/mL	5.1 – 20.0 ng/mL	> 20.0 ng/mL
APPARENTLY HEALTHY					
All Normals	240	228	12	0	0
BENIGN CONDITIONS					
Benign Lung Disease	75	71	4	0	0
CHF	40	30	10	0	0
Benign Liver Disease	40	38	2	0	0
Benign Kidney Disease	40	4	32	4	0
CANCER					
Lung Cancer	120	47	36	24	13
Bladder Cancer	40	17	3	12	8
Breast Cancer	40	32	6	1	1
Cervical Cancer	40	24	13	3	0
Esophageal SC Cancer	40	13	18	7	2
Gastrointestinal Cancer	40	25	10	4	1
Head and Neck Cancer	40	30	9	1	0
Prostate Cancer	40	37	1	2	0
Ovarian Cancer	40	23	10	5	2

CHF: Congestive heart failure; SC: Squamous cell

In this study 95% of the healthy subjects had a CYFRA 21-1 assay value at or below 1.8 ng/mL. It is recommended that each laboratory establish its own reference value for the population of interest.

MONITORING THE COURSE OF DISEASE IN PATIENTS DIAGNOSED WITH LUNG CANCER

The effectiveness of the CYFRA 21-1 EIA as an aid in monitoring the course of disease in lung cancer patients was determined through a retrospective clinical study. Changes in CYFRA 21-1 levels in serial serum samples collected from a tertiary cancer center were compared to changes in disease status. A study involving 100 patients was undertaken with a total of 314 observation pairs with an average number of 4.1 observations per patient. For the 100 lung cancer cases, 95 were classified as non-small cell lung cancer (NCSLC) and 5 were classified as small cell

lung cancer (SCLC). 90 of the NSCLC were further classified as adenocarcinoma (68), squamous cell carcinoma (19), and large cell carcinoma (3). 79 of the 100 lung cancer cases had staging information as shown in the following table. In this study, only 5 patients had Stage I or II disease, the performance of CYFRA 21-1 has not been adequately assessed in this subpopulation.

	Number of Patients
Stage I	2
Stage II	3
Stage III	36
Stage IV	38
Total (with Stage Information)	**79**
Unknown	17
Unstaged	4
Total Lung Cancer Cases	**100**

A positive change in CYFRA 21-1 was defined as a measurable increase in the value that was at least 50% greater than the previous value of the test. Observation pairs with both values below the normal reference range of 1.8 ng/mL were defined as no significant change. This level of change takes into account the variability of the assay and the biological variability (14).

Forty-six percent (46%) or 39/85 of the patient samples with a positive change correlated with the disease progression while eighty-seven percent (87%) or 200/229 of the patient serial samples with no significant change in CYFRA 21-1 value correlated with no progression. The total concordance was seventy-six percent (76%) or 239/314. The following table presents the data in a 2 x 2 format.

Change in Disease State per Sequential Pair

Increase in CYFRA 21-1 concentration	**Progression**	**No Progression**	**Total**
>50%	39	29	68
≤50%	46	200	246
Total	**85**	**229**	**314**

Clinicians may wish to use other percent changes in CYFRA 21-1 concentration to reflect their preferences in the trade-off between sensitivity and specificity.

The following table shows the resulting sensitivities and specificities of the CYFRA 21-1 EIA at various percent changes in CYFRA 21-1 EIA concentrations, together with the positive predictive values (PPV) and negative predictive values (NPV) for the population tested (85 sequential pairs from patients with disease progression and 229 sequential pairs from patients with no progression).

- Sensitivity is represented as the proportion of patients with disease progression that had elevated CYFRA 21-1
- Specificity is represented as the proportion of patients without disease progression that had no elevation in CYFRA 21-1
- PPV is represented as the proportion of patients with elevated CYFRA 21-1 that had disease progression
- NPV is represented as the proportion of patients with no elevation in CYFRA 21-1 that did not have disease progression

Percent Increase in CYFRA 21-1 Concentration (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
30	52.9	84.3	55.6	82.8
40	48.2	85.6	55.4	81.7
50	45.9	87.3	57.4	81.3
60	44.7	88.2	58.5	81.1
70	43.5	89.5	60.7	81.0

PERFORMANCE CHARACTERISTICS

Precision

A precision study was performed for the CYFRA 21-1 EIA per the Clinical and Laboratory Standards Institute (CLSI) guideline EP5-A2 (21). A panel of four serum samples was assayed, using two lots of reagents, in replicates of two, at two separate times per day for 20 days, at two different laboratories. The combined data from the two laboratories is summarized below:*

Sample	Reagent Lot	N	Mean conc. ng/mL	Within-run SD (ng/mL)	Within-run CV %	Total SD (ng/mL)	Total CV %
1	1	160	2.69	0.10	3.7	0.16	6.1
	2	160	2.91	0.15	5.1	0.25	8.4
2	1	160	7.28	0.31	4.3	0.50	6.9
	2	160	7.63	0.31	4.1	0.56	7.3
3	1	160	17.4	0.42	2.4	0.92	5.3
	2	160	18.6	0.48	2.6	0.91	4.9
4	1	160	33.4	0.88	2.6	1.62	4.9
	2	160	35.7	1.37	3.8	1.99	5.6

**Representative data; results in individual laboratories may vary from these data.*

The total precision determined for the CYFRA 21-1 EIA was found to be < 8.6% CV.

Detection and quantitation limit

The Limit of Detection of the CYFRA 21-1 EIA Kit was determined to be 0.12 ng/mL. The NCCLS CLSI guideline EP17-A (22) was used to design the LoD experiments. The limit of detection (LoD) corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration of CYFRA 21-1 antigen that can be distinguished from zero.

The Limit of Quantitation of the CYFRA 21-1 EIA Kit was determined to be 0.21 ng/mL. The limit of quantitation (LoQ) corresponds to the lowest amount of analyte in a sample that can be accurately quantitated with the highest allowable imprecision of 17.78%.

Recovery

The CYFRA 21-1 EIA assay mean recovery is 100 ± 20%. A study was performed where dilutions of an antigen solution with known concentrations of CYFRA 21-1 were added to normal human serum samples. The concentration of CYFRA 21-1 was determined using the CYFRA 21-1 EIA assay and the resulting percent recovery was calculated. Representative data from this study is summarized in the following table*:

Sample	Endogenous Assay Value (ng/mL)	CYFRA 21-1 Antigen Added (ng/mL)	Observed CYFRA 21-1 Assay Value (ng/mL)	Percent Recovery** %
1	0.5	2	2.3	93
		5	5.4	100
		16	15.4	91
		38	39.9	103
2	0.5	2	2.5	99
		5	5.2	96
		16	16.4	97
		38	39.6	102
3	0.6	2	2.6	102
		5	5.4	99
		16	16.1	95
		38	42.0	108
4	0.5	2	2.4	95
		5	5.3	98
		16	17.6	104
		38	43.1	111
5	0.5	2	2.4	96
		5	5.4	100
		16	17.1	101
		38	39.2	101

**Representative data; results in individual laboratories may vary from these data.*

***% Recovery=Observed CYFRA 21-1 Concentration (ng/mL)/Endogenous CYFRA 21-1 Conc. (ng/mL) + CYFRA 21-1 Added (ng/mL)*

The average recovery across the four separate spiked concentrations shown above was found to be 100%.

High Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. No high dose hook effect was observed in samples up to 1100 ng/mL CYFRA 21-1 antigen.

Dilution Linearity

A study was conducted for the CYFRA 21-1 EIA modeled after the CLSI guideline EP6-A (23). Serum samples with elevated CYFRA 21-1 values were diluted with CYFRA 21-1 Calibrator A (zero). The CYFRA 21-1 concentration was determined for each dilution and the percent (%) recovery was calculated. Representative data from this study is summarized in the following table*.

Sample	Final Dilution Factor	Obtained Value (ng/mL)	Expected Value (ng/mL)	Percent Recovery** (%)
A	Undiluted	42.400	42.400	100.0
	1:1.25	35.969	33.920	106.0
	1:2.5	17.540	16.960	103.4
	1:5	8.513	8.480	100.4
	1:10	4.078	4.240	96.2
	1:20	2.165	2.120	102.1
	1:40	1.010	1.060	95.3
	1:100	0.416	0.424	98.1
B	Undiluted	44.446	44.446	100.0
	1:1.25	36.524	35.557	102.7
	1:2.5	17.967	17.778	101.1
	1:5	8.292	8.889	93.3
	1:10	3.855	4.445	86.7
	1:20	2.090	2.222	94.0
	1:40	1.013	1.111	91.2
	1:100	0.494	0.444	111.1
C	Undiluted	43.511	43.511	100.0
	1:1.25	36.738	34.809	105.5
	1:2.5	18.202	17.404	104.6
	1:5	8.606	8.702	98.9
	1:10	4.094	4.351	94.1
	1:20	2.164	2.176	99.5
	1:40	1.005	1.088	92.4
	1:100	0.399	0.435	91.7

**Representative data; results in individual laboratories may vary from these data.*

***% Recovery= CYFRA 21-1 Concentration obtained x100 / Concentration expected*

The nonlinearity calculated by weighted polynomial regression is ≤ 10% across the measurement range of 0.5 to 50.0 ng/mL.

Analytical Specificity

The CYFRA 21-1 EIA assay mean assay specificity is 100 ± 15%. Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera. The CLSI guideline EP7-A (24) was used to design the interference experiments. The following substances and concentrations were tested and found not to interfere with the test.

Endogenous serum interferences	Test Concentration
Triglycerides	30 mg/mL
Billirubin	0.2 mg/mL
Hemoglobin	5 mg/mL
Total Protein	120 mg/mL
Chemotherapeutic drug interferences	**Test Concentration**
Carboplatin	500 µg/mL
Cisplatin	165 µg/mL
Dexamethasone	10 µg/mL
Doxorubicin	1.16 µg/mL
Leucovorin	2.68 µg/mL
Methotrexate	45 µg/mL
Paclitaxel	3.5 ng/mL

Potentially interfering clinical conditions

The CYFRA 21-1 EIA assay was evaluated using specimens with HAMA and Rheumatoid Factor (RF) to further assess the assay specificity. Six specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with CYFRA 21-1 antigen spiked into each specimen at approximately 5 and 25 ng/mL. Mean recovery results are summarized in the following table.*

Clinical condition	Number of specimens	Mean % recovery
HAMA	6	98
RF	5	101

**Representative data; results in individual laboratories may vary from these data.*

WARRANTY

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. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression of specific cytokeratins in normal epithelia, tumors and cultured cells. *Cell 1982;31:11-24*
2. Hesse M, Magin TM, Weber K. Genes for intermediate filament proteins and the draft sequence of the human genome: Novel Keratin genes and a surprisingly high number of pseudogenes related to keratin genes 8 and 18. *J Cell Sci 2001;114:2569-2575*
3. Bodenmüller H, Ofenloch-Hähnle B, Lane EB, Dessauer A, Böttger V, Donié F. Lung Cancer associated Keratin 19 fragments: Development and Biochemical Characterization of the new Serum Assay Enzymun-Test CYFRA 21-1. *Int J Biol Markers 1994;9:75-81*
4. Bodenmüller H. The Biochemistry of CYFRA 21-1 and other cytokeratin-tests. *Scand J Clin Lab Invest Suppl 1995;221:60-66*
5. Stieber P, Dienemann H, Hasholzner U, Müller C, Poley S, Hofmann K, Fateh-Moghadam. Comparison of Cytokeratin Fragment 19 (CYFRA 21-1) Tissue Polypeptide antigen (TPA) and Tissue Polypeptide Specific Antigen (TPS) as Tumor Markers in Lung Cancer. *Eur J Clin Chem Clin Biochem 1993;31:689-694*
6. Bodenmüller H, Donié F, Kaufmann M, Banauch D, The tumor markers TPA, TPS, TPAcyk and CYFRA 21-1 react differently with the keratins 8, 18 and 19. *Int J Biol Markers 1994;9:70-74.*
7. Böttger V, Stasiak PC, Harrison DL, Mellerick DM, Lane EB. Epitope mapping of monoclonal antibodies to keratin 19 using keratin fragments, synthetic peptides and phage peptide libraries. *Eur J Biochem 1995;231(2):475-85.*
8. Stigbrand T et al. Epitope specificity of 30 monoclonal antibodies against cytokeratin antigens: the ISOBM TD5-1 Workshop. *Tumor Biol 1998;19(2): 132-52.*
9. Stieber P, Hasholzner U, Bodenmüller H, Nagel D, Sunder-Plassmann L, Dienemann H, Meier W, Fateh-Moghadam A. CYFRA 21-1. A new marker in lung cancer. *Cancer 1993;72(3):707-13.*

10. Ebert W, Dienemann H, Fateh-Moghadam A, Scheulen M, Konietzko N, Schleich T, Bombardieri E. Cytokeratin 19 fragment CYFRA 21-1 compared with carcinoembryonic antigen, squamous cell carcinoma antigen and neuron-specific enolase in lung cancer. Results of an international multicentre study. *Eur J Clin Chem Clin Biochem 1994;32(3):189-99.*
11. Stieber P, Zimmermann A, Reinmiedl J, Müller C, Hoffmann H, Dienemann H. CYFRA 21-1 in the early diagnosis of recurrent disease in non small cell lung carcinomas (NSCLC). *Anticancer Res 1999;19(4A): 2665-8.*
12. Pujol JL, Molinier O, Ebert W, Daurès JP, Barlesi F, Buccheri G, Paesmans M, Quoix E, Moro-Sibilot D, Szturmowicz M, Bréchot JM, Muley T, Grenier J. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. *Br J Cancer 2004;90(11):2097-105.*
13. Stieber P, Dienemann H, Hasholzner U, Fabricius PG, Schambeck C, Weinzierl M, Poley S, Samtleben W, Hofmann K, Meier W, et al. Comparison of CYFRA 21-1, TPA and TPS in lung cancer, urinary bladder cancer and benign diseases. *Int J Biol Markers 1994;9(2):82-8.*
14. Trapé J, Perez de Olaguer J, Buxó J, López L. Biological Variation of Tumor Markers and Its Application in the Detection of Disease Progression in Patients with Non-Small Cell Lung Cancer. *Clin Chem 2005;51:219-222*
15. Volmer RT, Govindan R, Graziano SL, Gamble G, Garst J, Kelley MJ, Christenson RH. Serum CYFRA 21-1 in Advanced Stage Non-Small Cell Lung Cancer: An early measure of Response. *Clin Cancer Res 2003; 9:1728-1733.*
16. Holdenrieder S, Stieber P, von Pawel J, Raith H, Nagel D, Feldmann K, Siedel D. Early and Specific Prediction of the Therapeutic Efficacy in Non-Small Cell Lung Cancer Patients by Nucleosomal DNA and Cytokeratin-19 Fragments. *Ann. N.Y. Acad. Sci. 2006; 1075:244-257.*
17. Holdenrieder S, von Pawel J, Dankelmann E, Duell T, Faderl B, Markus A, Siakavara M, Wagner H, Feldmann K, Hoffmann H, Raith H, Nagel D, Stieber P. Nucleosomes, ProGRP, NSE, CYFRA 21-1, and CEA in monitoring First-Line Chemotherapy of Small Cell Lung Cancer. Clin Cancer Res:2008; 14(23): 7813-7821.
18. Nakayama M, Satoh H, Ishikawa H, Fujiwara M, Kamma H, Ohtsuka M, Sekizawa K. Cytokeratin 19 Fragment in Patients With Nonmalignant Respiratory Diseases. *Chest: 2003; 123: 2001-2006.*
19. US Department of Labor, Occuptional Safety and Health Administration, 29 CFR Part 1910.1030, Occupational Exposure to Blood Borne Pathogens.
20. US Department of Health and Human Services: Biosafety in Microbiological and Biomedical Laboratories: 4th Edition Washington DC: US Government Printing Office May, 1999.

21. Clinical and Laboratory Standards Institute (CLSI), Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline – Second Edition. EP5-A2 (2004).
22. Clinical and Laboratory Standards Institute (CLSI), Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. EP17-A (2004).
23. Clinical and Laboratory Standards Institute (CLSI), Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. EP6-A.
24. Clinical and Laboratory Standards Institute (CLSI), Interference Testing in Clinical Chemistry, Approved Guideline, EP7-A.

FUJIREBIO®
Diagnostics, Inc.

CanAg® is a registered trademark of Fujirebio Diagnostics AB

Fujirebio Diagnostics AB
Elof Lindälvs gata 13
SE-414 58 Göteborg
Sweden
Phone + 46 31 85 70 30
Fax + 46 31 85 70 40
info@fdab.com
www.fdab.com

CYFRA 21-1 EIA Prod. No. 211-10, 2011-05. F5963, r1