

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



CYFRA 21-1 EIA

REF

211-10

IVD

CE

Instructions for use. 2009-06

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

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

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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 ОБОЗНАЧЕНИЯ



Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Houdbaar tot/Holdbar til/
Ρουζιτελνή до/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 number/Lotnummer/
Číslo šarže/Αριθμός Παρτίδας/
Código do lote/Sarzszzám
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 produkcji/Pagaminimo data/
Дата производства

REF

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



Manufacturer/Hersteller/Fabricante/
Fabbicante/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/Indeholder tilstrækkeligt
til "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 sani-
tario para diagnóstico in vitro/
Dispositivo medico-diagnostico in vitro/
Dispositif médical de diagnostic in vitro/
Medisch hulpmiddel voor in-vitro
diagnostiek/Medicinsk udstyr til in
vitro-diagnostik/In Vitro diagnostický
zdravotnický prostředek /
In Vitro Διαγνωστικό Ιατροτεχνολογικό
προϊόν/Dispositivo médico para
diagnóstico in vitro/In vitro
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 rozmezi od do/
Περιορισμοί θερμοκρασίας/
Limites de temperatura/
Hőmérséklettartomány/
Temperaturbegränsning/
Przestrzegać zakresu temperatury/
Temperatūriņai 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
roužití/Συμβουλευτείτε τις οδηγίες
χρήσης/Consulte as instruções de
utilização/Nézze meg a Használati
utasítást/Se bruksanvisning/Sprawdź
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

CONT

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

ORIG **MOU**

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

ORIG **HUM**

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



Reconstitute with/Rekonstituieren mit/
Reconstituer con/Riconstituito con/
A reconstituer avec/Reconstituir com/
Rekonstituera med

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 quantitative determination of soluble cytokeratin 19 fragments in human serum.

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 eg. bladder cancer (13). The most important indication for CYFRA 21-1 is for monitoring the course of disease in non-small cell lung cancer (NSCLC), (11).

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) labelled 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 colour will develop if antigen is present. The intensity of the colour development is proportional to the amount of CYFRA 21-1 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 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
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MICROPLA

Streptavidin 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 aluminum pouch, containing desiccant. Reseal carefully to keep dry.

CAL	CYFRA 21-1	A
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CYFRA 21-1 Calibrator A	1 x 8 mL	2–8°C until expiry date stated on the vial
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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.

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			
<table border="1"><tr><td>CAL</td><td>CYFRA 21-1</td><td>B</td></tr></table>	CAL	CYFRA 21-1	B	1 x 1 mL	
CAL	CYFRA 21-1	B			
<table border="1"><tr><td>CAL</td><td>CYFRA 21-1</td><td>C</td></tr></table>	CAL	CYFRA 21-1	C	1 x 1 mL	
CAL	CYFRA 21-1	C			
<table border="1"><tr><td>CAL</td><td>CYFRA 21-1</td><td>D</td></tr></table>	CAL	CYFRA 21-1	D	1 x 1 mL	
CAL	CYFRA 21-1	D			
<table border="1"><tr><td>CAL</td><td>CYFRA 21-1</td><td>E</td></tr></table>	CAL	CYFRA 21-1	E	1 x 1 mL	
CAL	CYFRA 21-1	E			
<table border="1"><tr><td>CAL</td><td>CYFRA 21-1</td><td>F</td></tr></table>	CAL	CYFRA 21-1	F	1 x 1 mL	
CAL	CYFRA 21-1	F			

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			
<table border="1"><tr><td>CONTROL</td><td>CYFRA 21-1</td><td>1</td></tr></table>	CONTROL	CYFRA 21-1	1	1 x 1 mL	
CONTROL	CYFRA 21-1	1			
<table border="1"><tr><td>CONTROL</td><td>CYFRA 21-1</td><td>2</td></tr></table>	CONTROL	CYFRA 21-1	2	1 x 1 mL	
CONTROL	CYFRA 21-1	2			

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.

Component	Quantity	Storage and stability after first use
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BIOTIN	Anti-CYFRA 21-1
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Biotin Anti-CYFRA 21-1	1 x 15 mL	2–8°C until expiry date stated on the vial
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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
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Tracer, HRP Anti-CYFRA 21-1	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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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
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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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Contains 0.12 M hydrochloric acid. Ready for use.

Component	Quantity	Storage and stability after first use
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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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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 TMBHRP-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.
- 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 serum samples 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.
- 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.

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 gently before analysis.

Note: Mixing of samples using roller mixers must be limited to a maximum of 1 minute with a maximum speed of 16 rpm. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds. Samples that contain gross particulates should be centrifuged at 10.000 x g for 10 minutes prior to use to eliminate any particulate matter that may have developed from the thawing process.

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 deionised water

For reconstitution of CYFRA 21-1 Calibrators, CYFRA 21-1 Controls and for preparation of diluted wash solution.

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 after thawing. *Mixing of samples using roller mixers must be limited to a maximum of 1 minute with a maximum speed of 16 rpm. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds. 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 TMBHRP-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.

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 standing 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. *Mixing of calibrators using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds.*

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 standing for at least 15 minutes to reconstitute and mix gently before use. **NOTE:** The range of the controls is stated on the labels. *Mixing of controls using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds.*

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 deionised water to give a buffered Wash Solution.

Preparation of reagents	Stability of prepared reagent
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):

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 Antibody 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 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. Mixing of samples using roller mixers must be limited to a maximum of 1 minute with a maximum speed of 16 rpm. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds. 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:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	1 st Unk				
B	Cal A	Cal E	1 st 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					

- Add 100 μ L of Antibody Solution to each well using a 100 μ L 8-channel precision pipette (or a 100 μ L precision pipette). Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over.
- Incubate the plate for 1 hour (\pm 5 min) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker.
- After the incubation aspirate and wash each strip 6 times.
- Add 100 μ L 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.
- Incubate for 30 min (\pm 5 min) at room temperature with constant shaking. Avoid exposure to direct sunlight.
- 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 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.

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	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">CAL</td> <td style="width: 50%;">CYFRA 21-1</td> </tr> <tr> <td colspan="2" style="text-align: center;">B, C, D, E, F</td> </tr> </table>	CAL	CYFRA 21-1	B, C, D, E, F		Add 1 mL of distilled or deionised water to each vial. Allow to stand for at least 15 minutes and mix gently. NOTE: The exact concentration of each calibrator is stated on the label. Mixing of calibrators/controls using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds.																										
	CAL	CYFRA 21-1																														
B, C, D, E, F																																
Prepare CYFRA 21-1 Controls	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">CONTROL</td> <td style="width: 50%;">CYFRA 21-1</td> </tr> <tr> <td colspan="2" style="text-align: center;">1, 2</td> </tr> </table>	CONTROL	CYFRA 21-1	1, 2																												
CONTROL	CYFRA 21-1																															
1, 2																																
Prepare Wash Solution	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">WASHBUF</td> <td style="width: 50%;">25X</td> </tr> </table>	WASHBUF	25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionised water.																												
WASHBUF	25X																															
Prepare Antibody Solution	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">CONJ</td> <td style="width: 50%;">Anti-CYFRA 21-1</td> </tr> </table>	CONJ	Anti-CYFRA 21-1	Mix 50 µL of Tracer, HRP Anti-CYFRA 21-1 with 1 mL of Biotin Anti-CYFRA 21-1 per strip:																												
	CONJ	Anti-CYFRA 21-1																														
<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">BIOTIN</td> <td style="width: 50%;">Anti-CYFRA 21-1</td> </tr> </table>	BIOTIN	Anti-CYFRA 21-1																														
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<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left;">No. of Strips</th> <th style="text-align: left;">Tracer, HRP Anti-CYFRA 21-1 (µL)</th> <th style="text-align: left;">Biotin Anti-CYFRA 21-1 (mL)</th> </tr> </thead> <tbody> <tr><td style="text-align: center;">1</td><td style="text-align: center;">50</td><td style="text-align: center;">1</td></tr> <tr><td style="text-align: center;">2</td><td style="text-align: center;">100</td><td style="text-align: center;">2</td></tr> <tr><td style="text-align: center;">3</td><td style="text-align: center;">150</td><td style="text-align: center;">3</td></tr> <tr><td style="text-align: center;">4</td><td style="text-align: center;">200</td><td style="text-align: center;">4</td></tr> <tr><td style="text-align: center;">5</td><td style="text-align: center;">250</td><td style="text-align: center;">5</td></tr> <tr><td style="text-align: center;">6</td><td style="text-align: center;">300</td><td style="text-align: center;">6</td></tr> <tr><td style="text-align: center;">7</td><td style="text-align: center;">350</td><td style="text-align: center;">7</td></tr> <tr><td style="text-align: center;">8</td><td style="text-align: center;">400</td><td style="text-align: center;">8</td></tr> <tr><td style="text-align: center;">9</td><td style="text-align: center;">450</td><td style="text-align: center;">9</td></tr> </tbody> </table>			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
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																														

				9 400
				10 500
				11 550
				12 600
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 roller mixers must be limited to a maximum of 1 minute with a maximum speed of 16 rpm. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds.	
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%.
- 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.

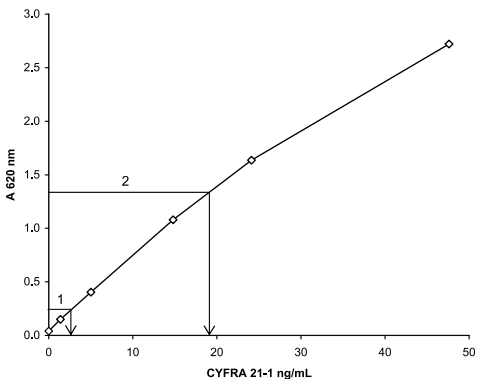
For automatic calculation of CYFRA 21-1 results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 ng/mL.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 ng/mL.
- Quadratic curve fit method. Calibrator A should be included in the curve with the value 0 ng/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 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.

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 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. acute pneumonia, tuberculosis, liver diseases and renal failure. 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. Contamination of the sample with saliva may cause falsely elevated levels of CYFRA 21-1. 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 roller mixers must be limited to a maximum of 1 minute with a maximum speed of 16 rpm. Mixing of samples using electric vibration mixers (Vortex) must be limited to a maximum of 5 seconds. If aliquoted choose the right sized tube i.e. limit the empty space in the tube.*

EXPECTED VALUES

The level of CYFRA 21-1 was determined in 497 blood donors. The mean value was 0.7 ng/mL and the median value was 0.6 ng/mL.

95% of the blood donors had CYFRA values below 1.6 ng/mL.

It is recommended that each laboratory establish its own reference value for the population of interest.

PERFORMANCE CHARACTERISTICS

Precision

Total precision was determined according to NCCLS guideline EP5-A2 (16) using four levels of frozen pooled human serum containing added CYFRA 21-1. Each sample was randomly pipetted in duplicates and analysed twice each day over 20 days i.e. 40 runs with 40 different templates by three different technicians using 2 different CYFRA 21-1 EIA kit batches.

Sample	Replicates	Mean µg/L	Within-run SD (ng/mL)	Within-run CV %	Between SD (ng/mL)	Between CV %
CYFRA 21-1 1	80	2.7	0.1	2.4	0.1	3.9
CYFRA 21-1 2	80	7.2	0.1	1.7	0.2	3.2
CYFRA 21-1 3	80	17.7	0.5	2.6	0.5	3.1
CYFRA 21-1 4	80	35.7	1.0	2.7	1.1	3.0

The total precision determined for the CYFRA 21-1 EIA was found to be $\leq 6\%$ CV.

Detection limit

The limit of detection of the CYFRA 21-1 EIA assay is ≤ 0.5 ng/mL.

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 NCCLS guideline EP17-A (17) was used to design the LoD experiments. A study was conducted where CYFRA 21-1 Calibrator A (zero) and 4 samples from healthy subjects diluted to 0.2 ng/mL with Sample Diluent was tested in replicates of 24 per run in 4 runs on two separate days. The LoD was calculated as follows:

$$\text{LoD (ng/mL)} = 0.2 \text{ ng/mL} \times (1.65 \times \text{SD}_0 + 1.65 \times \text{SD}_{0.2}) / (\text{OD}_{0.2} - \text{OD}_0)$$

The Limit of Detection of the CYFRA 21-1 EIA Kit was found to be < 0.1 ng/mL.

Functional sensitivity

The functional sensitivity of the CYFRA 21-1 EIA assay is ≤ 0.5 ng/mL.

The functional sensitivity is expressed as the concentration of an analyte at which the CV is 20%. The NCCLS guideline EP5-A2 (16) was used to design the experiments for determination of functional sensitivity. A study was conducted where a six member sensitivity panel was tested in replicates of 2 in 2 runs on twenty separate days with two lots of reagents. The functional sensitivity determined for the CYFRA 21-1 EIA was found to be < 0.2 ng/mL.

Recovery

The CYFRA 21-1 EIA assay mean recovery is $100 \pm 20\%$.

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

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

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

**% Recovery = $\frac{\text{Observed CYFRA 21-1 Concentration (ng/mL)}}{\text{Endogenous CYFRA 21-1 Conc. (ng/mL) + CYFRA 21-1 Added (ng/mL)}}$

High Dose Hook

No high dose hook effect was observed for samples containing up to 1100 ng/mL CYFRA 21-1 antigen.

Dilution Linearity

The CYFRA 21-1 EIA assay mean dilution linearity is $100 \pm 20\%$.

A study was conducted for the CYFRA 21-1 EIA modeled after the NCCLS (CLSI) guideline EP6-A (18). 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	43.9	43.9	100
	1:1.25	34.8	35.1	99
	1:1.7	26.0	26.3	99
	1:2	21.5	21.9	98
	1:2.5	17.1	17.5	97
	1:5	9.0	8.8	102
	1:10	4.3	4.4	99
	1:20	2.2	2.2	102
B	Undiluted	36.9	36.9	100
	1:1.25	28.6	29.5	97
	1:1.7	21.1	22.1	96
	1:2	18.3	18.4	99
	1:2.5	14.5	14.8	98
	1:5	6.9	7.4	93
	1:10	3.2	3.7	86
	1:20	1.5	1.8	81
C	Undiluted	43.2	43.2	100
	1:1.25	34.1	34.6	99
	1:1.7	25.4	25.9	98
	1:2	21.0	21.6	97
	1:2.5	16.5	17.3	96
	1:5	7.8	8.6	91
	1:10	3.8	4.3	87
	1:20	1.8	2.2	83

Sample	Final Dilution Factor	Obtained Value (ng/mL)	Expected Value (ng/mL)	Percent Recovery** (%)
D	Undiluted	43.7	43.7	100
	1:1.25	33.1	35.0	95
	1:1.7	25.9	26.2	99
	1:2	21.4	21.9	98
	1:2.5	16.6	17.5	95
	1:5	7.9	8.7	90
	1:10	3.8	4.4	86
	1:20	1.8	2.2	80
E	Undiluted	39.6	39.6	100
	1:1.25	30.6	31.7	97
	1:1.7	23.6	23.8	99
	1:2	19.7	19.8	100
	1:2.5	15.2	15.8	96
	1:5	7.3	7.9	92
	1:10	3.5	4.0	88
	1:20	1.6	2.0	82

Average recovery across the five diluted samples shown above was found to be 94%.

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

**% Recovery= CYFRA 21-1 Concentration obtained x Dilution factor/Undiluted CYFRA 21-1 Concentration.

Analytical Specificity

The CYFRA 21-1 EIA assay mean assay specificity is $100 \pm 15\%$.

Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera. The NCCLS guideline EP7-A (19) 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.

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