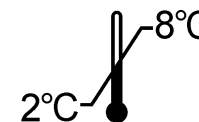




# CYFRA 21-1



ELSA-CYFRA-



<p><b>Trousse pour le dosage immunoradiométrique du CYFRA 21-1 dans le sérum humain</b> <b>Pour diagnostic In Vitro</b></p>	<p><b>Kit for the immunoradiometric assay of CYFRA 21-1 in human serum</b> <b>For In Vitro diagnostic use</b></p>	<p><b>Immunoradiometrischer Test zur quantitativen Bestimmung von CYFRA 21-1 in Humanserum</b> <b>Zur In Vitro Diagnostik</b></p>																																																
<p><b>Kit per il dosaggio immunoradiometrico del CYFRA 21-1 nel siero umano</b> <b>Per uso diagnostico In Vitro</b></p> <p>Contenuto del kit :</p> <table border="0"> <tr><td>ELSA</td><td>2 x 24 tubes</td></tr> <tr><td>Traceur ≤ 277,5 kBq</td><td>1 x 15 mL</td></tr> <tr><td>Calibrateur 0</td><td>1 x 4 mL</td></tr> <tr><td>Calibrateurs 1 – 4</td><td>4 x 1 mL</td></tr> <tr><td>Contrôle</td><td>1 x 1 mL</td></tr> <tr><td>Tween 20</td><td>1 x 10 mL</td></tr> <tr><td>Sachet plastique</td><td>1</td></tr> <tr><td>Notice d'utilisation</td><td>1</td></tr> </table> <p><b>Attention:</b> Certains réactifs contiennent de l'azoture de sodium</p>	ELSA	2 x 24 tubes	Traceur ≤ 277,5 kBq	1 x 15 mL	Calibrateur 0	1 x 4 mL	Calibrateurs 1 – 4	4 x 1 mL	Contrôle	1 x 1 mL	Tween 20	1 x 10 mL	Sachet plastique	1	Notice d'utilisation	1	<p><b>Equipo inmunorradiométrico destinado a la determinación cuantitativa del CYFRA 21-1 en suero humano</b> <b>Para uso de diagnóstico In Vitro</b></p> <p>Contenido del equipo :</p> <table border="0"> <tr><td>ELSA tubes</td><td>2 x 24 tubes</td></tr> <tr><td>Tracer ≤ 277.5 kBq</td><td>1 x 15 mL</td></tr> <tr><td>Calibrator 0</td><td>1 x 4 mL</td></tr> <tr><td>Calibrators 1 – 4</td><td>4 x 1 mL</td></tr> <tr><td>Control</td><td>1 x 1 mL</td></tr> <tr><td>Tween 20</td><td>1 x 10 mL</td></tr> <tr><td>Plastic bag</td><td>1</td></tr> <tr><td>Instruction for use</td><td>1</td></tr> </table> <p><b>Warning:</b> Some reagents contain sodium azide</p>	ELSA tubes	2 x 24 tubes	Tracer ≤ 277.5 kBq	1 x 15 mL	Calibrator 0	1 x 4 mL	Calibrators 1 – 4	4 x 1 mL	Control	1 x 1 mL	Tween 20	1 x 10 mL	Plastic bag	1	Instruction for use	1	<p><b>Δοκιμασία για τον ραδιοανοσολογικό προσδιορισμό του CYFRA 21-1 στον ανθρώπινο ορό</b> <b>Για διαγνωστική χρήση in vitro</b></p> <p>Περιεχόμενα της τυποποιημένης συσκευασίας</p> <table border="0"> <tr><td>ELSA</td><td>2 x 24 Röhrrchen</td></tr> <tr><td>Tracer ≤ 277,5 kBq</td><td>1 x 15 mL</td></tr> <tr><td>0 - Kalibrator</td><td>1 x 4 mL</td></tr> <tr><td>Kalibratoren 1 – 4</td><td>4 x 1 mL</td></tr> <tr><td>Kontrolle</td><td>1 x 1 mL</td></tr> <tr><td>Tween 20</td><td>1 x 10 mL</td></tr> <tr><td>Plastikbeutel</td><td>1</td></tr> <tr><td>Gebrauchsinformation</td><td>1</td></tr> </table> <p><b>Achtung:</b> Einige Reagenzien enthalten Natriumazid</p>	ELSA	2 x 24 Röhrrchen	Tracer ≤ 277,5 kBq	1 x 15 mL	0 - Kalibrator	1 x 4 mL	Kalibratoren 1 – 4	4 x 1 mL	Kontrolle	1 x 1 mL	Tween 20	1 x 10 mL	Plastikbeutel	1	Gebrauchsinformation	1
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FRA

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	Explication des symboles	Explanation of symbols	Erläuterung der Symbole	Spiegazione dei simboli	Significado de los símbolos	Επεξήγηση των συμβόλων που	Significadodos símbolos	Wyjaśnienie symboli	Обяснение на символите
	Conforme aux normes européennes	European conformity	CE-Konformitätskennzeichnung	Conformita europea	Conformidad europea	European conformity	Conformidad com as normas europeias	Zgodne z normami europejskimi	Европейската съответствие
	T° limite de stockage	Storage temperature limitation	Limitierung der Lagertemperatur	Limiti per la temperatura di conservazione	Limites de temperatura de almacenamiento	Περιορισμός θερμοκρασίας φύλαξης	Limite da temperatura de armazenagem	Graniczna temperatura przechowywania	Ограничаване на температурата на съхранение
	N° de lot	Batch code	Chargencode	codice lotto	Código de lote	Κωδικός παρτίδας	Lote	Numer partii	Batch код
	Utiliser jusqu'au	Use by	Verwendbar bis	utilizzare entro	Consumir antes de	Ημερομ. λήξης	Utilizado por	Zużyć do	Използвайте от
	Consulter la notice d'utilisation	Consult operating instructions	Das Handbuch zu Rate ziehen	consultare le istruzioni per l'USO	Consultar las instrucciones de manejo o funcionamiento	Ανατρέξτε στις οδηγίες λειτουργίας	Consulte o manual de operações	Patrz dołączona ulotka	Консултирайте се с инструкциите за експлоатация
	Diagnostic In Vitro	In Vitro Diagnostic device	In-VitroDiagnostische Anwendung	Dispositivo Diagnostico In Vitro	Dispositivo de diagnóstico In Vitro	διαγνωστική συσκευή In Vitro	Dispositivo de diagnostico In Vitro	Diagnostyka In Vitro	Ин витро диагностично устройство
	Fabriqué par	Manufactured by	Hergestellt von	Prodotto da	Fabricado por	Κατασκευάζεται από την	Fabricado por	Wyprodukowane przez	Произведено от
	Référence	Catalogue number	Katalog Nr.	N. catalogo	Número de catálogo	Αριθμός καταλόγου	Número do catalogo	Wzorzec	Каталожен номер
	Nombre de tubes	Number of determinations	Anzahl der Bestimmungen	Numero di determinazioni	Número de determinaciones	Αριθμός προσδιορισμών	Número de determinações	Liczba probówek	Брой на определянията
	Tubes revêtus	Coated tubes	beschichtete Röhrchen	Provette coattate	Tubos recubiertos	Επιστρωμένα σωληνάκια	Tubos adsorvidos	Probówki powlekane	Обвити тръби
	Traceur radioactif	Radioactive tracer	Radioactiver Tracer	Tracciante radioattivo	Trazador radiactivo	Ραδιενεργός ιχνηθέτης	Marcador radioativo	Znacznik radioaktywny	радиоактивни трасирац
	Calibrateur	Calibrator	Kalibrator	Calibratore	Calibrador	Βαθμονομητής	Calibrador	Kalibrator	Калибратор
	Contrôle	Control	Kontrolle	Controllo	Control	Όρος ελέγχου	Controle	Kontrola	контрол
	Solution de lavage	Wash solution	Waschlotion	Soluzione di lavaggio	Solución de lavado	Διάλυμα πλύσης	Solução de lavagem	Roztwór płuczący	Wash решение

**FRA** Modifications par rapport à la version précédente :

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**ENG** Changes from the previous version:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**DEU** Änderungen gegenüber der Vorgängerversion:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**ITA** Modifiche rispetto alla versione precedente:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**SPA** Cambios desde la versión anterior:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**ELL** Αλλαγές από την προηγούμενη έκδοση:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**POR** Alterações em relação à versão anterior:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**POL** Zmiany w stosunku do poprzedniej wersji:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

**BUL** Промени от предишната версия:

4. CAL 4: 60 →63 ng/mL / CONTROL: 4 →3.5ng/mL

### 1. NAME AND INTENDED USE

ELSA-CYFRA 21-1 is a kit for the immunoradiometric assay of CYFRA 21-1 in human serum.  
The kit is intended for professional use.

### 2. INTRODUCTION

CYFRA 21-1 is a cytokeratin 19 fragment found in serum of cancer patients. Precise recognition of this fragment is made with two monoclonal antibodies (BM 19-21 and KS 19-1)\* which were obtained after immunisation of mice with MCF-7 cells.

Cytokeratin 19 (CK19) is a member of the intermediate filament group of proteins, whose physiological role remains unclear. It is an acid-type cytoplasmic protein, with a molecular weight of 40 000 D, expressed in simple epithelium. On the death of the cell, it is released into the serum in the form of soluble fragments.

In immunohistochemistry, CK19 is found in the cytoplasm of the epithelial tumor cell, including that of bronchial cancers. Preliminary clinical studies of bronchial cancer patients sera have shown that CYFRA 21-1 assay is useful in the diagnosis and follow-up of non-small cell lung carcinoma and particularly of squamous cell carcinoma of the lung.

### 3. PRINCIPLE

ELSA CYFRA 21-1 is a solid-phase sandwich immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites on the CYFRA 21-1 molecule, the first being coated on the ELSA solid phase while the second, radiolabeled with iodine 125, is used as a tracer.

CYFRA 21-1 molecules present in the calibrators or the samples to be tested are sandwiched between the two antibodies. Excess unbound tracer is easily removed during the procedure's washing step, and the ELSA retains only the adsorbed antibody/antigen/tracer antibody combination.

The amount of radioactivity bound to the ELSA is proportional to the amount of CYFRA 21-1 present at the beginning of the assay.

### 4. REAGENTS

Each kit contains enough reagents for 48 tubes. The expiry date is marked on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
<b>ELSA TUBES:</b> ready for use. Monoclonal anti-CYFRA 21-1 antibody coated on ELSA fixed in the bottom of the tube.	<b>CT</b>	2 traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
<b>ANTI-CYFRA 21-1 <sup>125</sup>I:</b> ready for use. <sup>125</sup> I anti-CYFRA 21-1 monoclonal antibody, buffer, bovine albumin, sodium azide, red dye, non-immunized mouse immunoglobulins. ≤ 277,5 kBq (≤ 7,5 µCi).	<b>TRACER</b>	1 15 mL vial	2-8°C until the expiry date.
<b>0 CALIBRATOR:</b> lyophilized. Bovine serum. Reconstitute to 4 mL with distilled water.	<b>CAL</b>	1 4 mL vial	2-8°C, 2 days after reconstitution, or -20°C for 1 month within the limits of the expiry date.
<b>CALIBRATORS:</b> lyophilized. Bovine serum, human CYFRA 21-1. 2 - 10 - 30 - 63 ng/mL** Reconstitute to 1 mL with distilled water.	<b>CAL</b>	4 1 mL vials	
<b>CONTROL:</b> lyophilized. Bovine serum, human CYFRA 21-1. 3.5 ng/mL**. Reconstitute to 1 mL with distilled water.	<b>CONTROL</b>	1 1 mL vial	
<b>TWEEN 20:</b> concentrated solution. Dilute 9 mL of Tween 20 in 3 liters of distilled water. Shake gently.	<b>TWEEN 20</b>	1 10 mL vial	2-8° C until the expiry date. After dilution, store in a capped container for 15 days (2-8°C) maximum, within the limits of the expiry date.
<b>PLASTIC BAG</b>		1	

(\*\*) The values shown above are only target values: the true value of each calibrator or control is shown on its label.

## 5. PRECAUTIONS FOR USE

### 5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

### 5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below:

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

### 5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Avoid any microbic contamination of the reagents or of the water used for washing.

Fully respect the incubation conditions and the washing instructions indicated.

## 6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum. If the test is to be carried out within 24 hours, the samples should be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots and deep frozen (-20°C) until needed.

### Dilution

Should elevated CYFRA 21-1 levels be suspected, the O Calibrator found in the kit is used for dilution.

It is recommended that disposable plastic tubes be used when carrying out the dilutions.

## 7. ASSAY PROCEDURE

### 7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 100µL, 300µL, 1mL and 4mL. Their calibration should be checked regularly.

Distilled water.

Disposable plastic tubes.

Vortex-type mixer.

Gamma scintillation counter calibrated for 125 iodine measurement.

### 7.2. Protocol

Calibrators, control and ELSA tubes must be brought to room temperature (18-25°C) at least 30 minutes before their use. The anti-CYFRA 21-1 tracer antibody must be stored at 2-8°C until immediately before use. Dispensing of the reagent into the ELSA tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:

O Calibrator group for the determination of non-specific binding,

Calibrator groups to establish the calibrator curve,

Control group for the control,

Sx groups for the test samples.

It is recommended to perform the assay in duplicate for the calibrator groups, the control and the samples.

Strictly respect the order in which reagents are to be added:

Dispense 100µL of calibrators, or control or samples to be assayed into the corresponding labelled ELSA tubes.

Add 300  $\mu\text{L}$  of  $^{125}\text{I}$  anti-CYFRA 21-1 to each ELSA tube. Tracer must be removed from 2-8°C storage immediately before use.

Mix each tube gently with a Vortex-type mixer.

Incubate for 20 h  $\pm$  2 h at 2-8°C.

Wash the ELSA tubes as follows:

Aspirate the contents of the tubes as completely as possible. Add 3.0 mL of washing solution to each tube, and re-empty. Repeat this process twice.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. As much as possible of the incubation and washing solutions must be removed. If manual aspiration is used, the tip of the aspirating device must be placed right at the bottom of the tube.

Measure the remaining radioactivity bound to the ELSA with a gamma scintillation counter.

## 8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

## 9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.

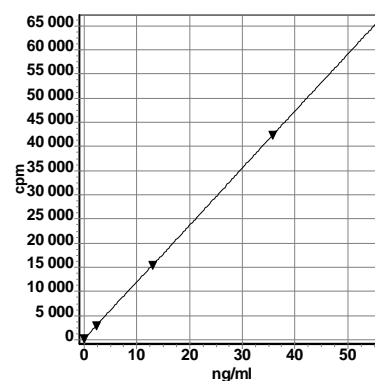
Draw up the calibrator curve by plotting the calibrators cpm against their concentrations.

Read the sample values directly from the curve, correcting the read value for the dilution factor if necessary.

The spline mathematical fitting model is recommended for calibration curve. Other fitting models may give slightly different results.

**Typical calibration curve** (example only): this data must not be substituted for results obtained in the laboratory.

Tube Groups	Mean cpm	Concentration ng/mL
Calibrator 0	189	0
Calibrator 1	2959	2.3
Calibrator 2	15456	13.0
Calibrator 3	42391	35.8
Calibrator 4	66130	56.0
Control	5657	4.6
Sample 1	5418	4.4
Sample 2	53489	45.2



## 10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate sample values beyond the last calibrator. Dilute the samples concerned and re-assay.

## 11. EXPECTED VALUES

A clinical study showed that 99.6% of presumed normal subjects ( $n = 250$ ) and 95% of benign pulmonary disease patients ( $n = 61$ ) had a CYFRA 21-1 level below 3.3 ng/mL.

No differences in sex, age and smoking habits were observed. These results are only given as an indication and it is recommended that each laboratory establish its own range of clinical values.

## 12. SPECIFIC CHARACTERISTICS OF THE ASSAY

### 12.1. Imprecision

This has been assessed using 2 pools of assay samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 10 different series.

Sample	Mean ng/mL	Within-run CV %	Between-run CV %
1	2.3	4.6	5.5
2	37.7	3.1	5.7

### 12.2. Recovery test

Known quantities of CYFRA 21-1 were added to human sera. The recovery percentages of CYFRA 21-1 in the samples ranged from 95 to 110%.

### 12.3. Dilution test

Ten samples with high levels were diluted, with the recovery percentages ranging from 85 to 105%.

#### 12.4. Detection limit

The detection limit is defined as being the smallest detectable concentration different from 0 with a probability of 95%. It has been assessed as being 0.05 ng/mL.

#### 12.5 Interference

No interference with bilirubin, haemoglobin, and triglycerides, measured up to respective concentrations of equal to 250 mg/L, 10 g/L, and 20 g/L, has been observed.

The immunoassay is protected against any human anti-mouse antibody (HAMA) interference by the addition of a protector to the tracer (non-specific mouse immunoglobulins). However, we can not guarantee that this protection is exhaustive.

#### ASSAY FLOW CHART

Tubes	Calibrators Control Samples μL	<sup>125</sup> I Anti- CYFRA 21-1 μL	Mix. Incubate 20 H ± 2 H at 2-8°C.  Wash 3 times.	Count
Calibrators	100	300		
Control or Samples	100	300		

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