RK-211CTACE181201

CYFRA 21.1 [I-125] IRMA KIT

(REF: RK-211CT and RK-211CT50)

The CYFRA 21.1 IRMA system provides a direct *in vitro* quantitative determination of Cytokeratin 19 fragments in human serum in the range of 0-60 ng/mL. Each kit contains material sufficient for 100 or 50 assay tubes, permitting the construction of one standard curve and the assay of 42 (RK-211CT) or 17 (RK-211CT50) unknowns in duplicates.

Introduction

Cytokeratin 19 is member of a family of more than 20 different cytokeratin polypeptides which form the intermediate filament structure of epithelial cells. This is an acid-type cytoplasmic protein with a molecular weight of 40000 Da. Cytokeratin filaments are poorly soluble but following proteolytic degradation, soluble fragments are formed and released into body fluids.

CYFRA 21.1 IRMA is an immunoassay that determines the concentration of Cytokeratin 19 fragments in serum. The determination is based on the use of two monoclonal antibodies: BM 19.21* and KS 19.1*, which recognize two different epitopes located in the C-terminal helical region of the molecule.

Elevated concentrations of Cytokeratin 19 fragments are seen in serum from patients with non-small cell lung cancer. The CYFRA 21.1 assay is not useful as a screening test. However, it is of aid in monitoring disease progression, treatment efficiency, and in the early detection of relapse and long-term follow up of patients.

Principle of method

The technology uses two monoclonal antibodies of high affinity in an immuno-radiometric assay (IRMA) system. The ^{125}I labelled signal-antibody (BM19.21*) binds to an epitope of the molecule spatially different from that recognized by the biotin-capture-antibody (KS19.1*). These antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a **capture antibody - antigen - signal antibody** complex, also referred to as a "sandwich".

During an overnight incubation at 2 - 8°C, the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. The reaction mixture is then discarded, the test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve, plotting binding values against a series of calibrators containing known amounts of CYFRA 21.1, the unknown concentration of patient samples can be determined.

*Fujirebio Diagnostics Inc. antibodies

Contents of the kit

1. One bottle of TRACER (11 mL), ready to use, containing < 980 kBq ^{125}I-anti-CYFRA 21.1 antibody and biotin-capture antibody in buffer with red dye and 0.1 % NaN_3.

2. One bottle (10 mL) of DILUENT, ready to use, equine serum with 0.1% NaN_3.

3. Six vials of STANDARDS S0-S5 (6 x 1 mL), freeze-dried, in equine serum (S0) and human serum (S1-S5) with 0.1% Kathon-CG. The concentrations of standards are specified in the quality certificate enclosed. *Assay calibration was performed using Fujirebio Diagnostics Inc. CYFRA 21.1IRMA.*

4. Two vials of CONTROL SERA (2x1 mL), freeze-dried, in human serum with 0.1% Kathon-CG. The concentrations of controls are specified in the quality certificate enclosed.

5. COATED TUBES, ready to use.

Reactive test tubes, 12x75 mm, packed in plastic boxes. (**RK-211CT**: 2 boxes, 2x50 pcs; **RK-211CT50**: 1 box, 1x50 pcs)

6. One bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN_3. *See Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

- common laboratory equipment
- 100 µL precision micropipette
- 100 µL repeating pipette
- 2000 µL repeating pipette or dispenser
- plastic foil to cover tubes
- absorbent tissue
- gamma-counter

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Before assaying, frozen samples should be thawed and gently mixed by inversion.

Hemolyzed and lipemic specimens may give false values and should be avoided.

Samples with a CYFRA 21.1 concentration higher than 60 ng/mL should be diluted with Diluent (D) and reassayed. Recommended dilution: 10-fold (450 µL D + 50 µL sample).

Preparation of reagents, storage

Store the reagents between 2-8°C. At this temperature each reagent is stable until the expiration date of the kit.

Tracer should be removed from 2-8°C storage immediately before use!

Reconstitute Standards and Controls with 1 mL distilled water before use. After 20 minutes, mix by inversion or vortex gently. ***For further use, please aliquot and keep frozen!***

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. After dilution, store at 2-8°C until the expiration date of the kit.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S0-S5), control serum and sample. Label two test tubes for total counts (T).
2. Pipette 100 µL of standards, controls and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
3. Pipette 100 µL of tracer into each tube. Mix gently the content of tubes.
4. Seal all tubes with a plastic foil. Incubate 20 ± 2 hours at 2-8°C.
5. Add 2.0 mL of wash buffer to each tube. Decant the content of tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
6. Return the tube-rack to an upright position and repeat step-5 two more times.
7. Count each tube for at least 60 seconds in a gamma counter.
8. Calculate the CYFRA 21.1 concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Tracer	**100**	**100**	**100**	**100**
Incubate overnight at 2-8°C				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}5} / C_{I\text{-}II} / M_x \text{ (cpm)} - S_0 \text{ (cpm)}}{T\text{(cpm)}} \times 100$$

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of CYFRA 21.1.

Determine the CYFRA 21.1 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.
Out of fitting programs applied for computerized data processing, spline fittings are recommended.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	CYFRA 21.1 ng/mL
T	304068		
S0	812	0.3	0
S1	4026	1.3	1.1
S2	10446	3.4	4.7
S3	25735	8.5	13
S4	52315	17.2	27
S5	99240	32.6	54
CI	11438	3.8	5.3
CII	31914	10.5	16.3

Performance characteristics

Specificity

The antibodies used in this assay guarantee a measurement completely specific for CYFRA 21.1.

Sensitivity

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:
Limit of Blank (LoB): 0.035 ng/mL
Limit of Detection (LoD): 0.24 ng/mL
For results under LoB, should report as "analyte not detected". For results between LoB and LoD, should report as "analyte detected", concentration < 0.24 ng/mL.

Precision and reproducibility

Three serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 12 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (ng/mL)	CV%	Mean (ng/mL)	CV%
1.11	4.16	1.31	9.25
4.37	7.48	5.52	8.77
21.27	7.05	26.3	6.98

Hook effect

No hook effect is observed for concentrations lower than 400 ng/mL.

Expected Values

It is recommended that each laboratory determine a reference range for its own patient population.

Serum samples from 200 presumably healthy blood donors were evaluated. All the samples measured had a concentration < 2.0 ng/mL.

Method comparison

The CYFRA 21.1 IRMA (Y) was compared to the Fujirebio Diagnostics Inc. CYFRA 21.1 IRMA (X) on 98 specimens ranging from 0 to 60 ng/mL. Linear regression analysis yielded the following results:

$Y = 1.0314X + 0.0762$ $R^2 = 0.9814$

Procedural notes

- The non-respect of the instructions in this insert may affect results significantly.
- Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
- **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

Limitations

- The CYFRA 21.1 assay should not be used as a cancer screening test.
- CYFRA 21.1 assay values greater than or equal to 2 ng/mL can be found in some healthy individuals and in patients with non-malignant conditions.
- A CYFRA 21.1 value below 2 ng/mL does not indicate the absence of residual cancer.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

Precautions

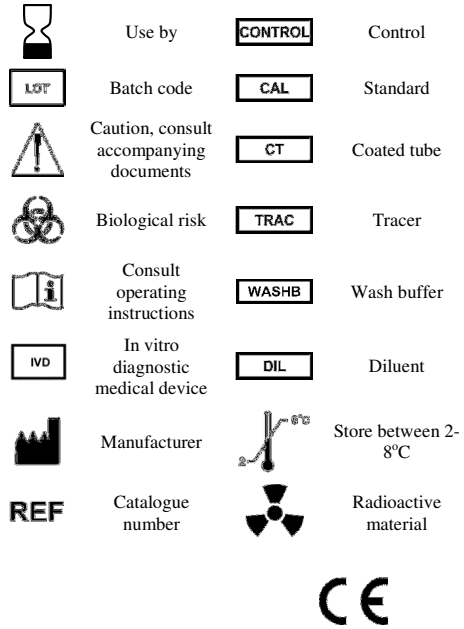
Radioactivity
This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard
Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 61 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.



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Legal note

CYFRA 21.1™ is a trade mark of Fujirebio Diagnostics Inc. (FDI). The present CYFRA 21.1 IRMA is based on the use of the KS 19.1 and BM 19.21 antibodies, which are available exclusively through FDI, and its licensed distributors.

FUJIREBIO Diagnostics, Inc.

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