CYFRA21-1 FIA Rapid Test Device

(Whole Blood/Serum/Plasma)

FI2-CA211-001

A rapid test for the quantitative detection of cytokeratin 19 fragments (CYFRA21-1) in whole blood, serum, or plasma with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000).
For professional in vitro diagnostic use only.

INTENDED USE

The Biopanda CYFRA21-1 FIA Rapid Test Device is intended for *in vitro* quantitative determination of cytokeratin 19 fragments (CYFRA21-1) in whole blood, serum or plasma as an aid to monitor the progression of lung cancer in patients during disease and treatment.

SUMMARY

Cytokeratin 19 fragment antigen (CYFRA21-1) is an epitope of a polypeptide that is released following cell death.[1] Cytokeratin 19 has an isoelectric pH of 5.2 and a molecular weight of 40 kDa and is present in intermediate fifilaments of the cytoskeletal structure of normal epithelium and in malignant epithelium.[2]

In malignant epidermal tumours, activated proteinases accelerate cell degeneration and cause the release of soluble CYFRA21-1 to tissues and body fluids. CYFRA21-1 has been reported to be the most sensitive tumour marker for nonsmall cell lung cancer (NSCLC) and appears to correlate with the development of disease.[3–5] Increased CYFRA21-1 levels have also been described in non-malignant diseases (i.e. pneumonia, sepsis)[6] and renal dysfunction.[7] Therefore, evaluation of renal function (i.e. by measuring serum creatinine levels) should be considered in cases of high CYFRA21-1 levels that are not consistent with the diagnostic and clinical characteristics of the patient.

PRINCIPLE

The Biopanda CYFRA21-1 Rapid Test Device detects CYFRA21-1 based on Fluorescence Immunoassay. During testing, the sample moves through the strip from sample pad to absorbent pad. The specimen which contains CYFRA21-1 conjugated with fluorescence particles in the label pad of test. The mixture migrates laterally along the membrane chromatographically by capillary action and reacts with the CYFRA21-1 antibody in test line region of nitrocellulose membrane. The concentration of CYFRA21-1 in the specimen correlates with the fluorescence signal intensity captured on the test line, which can be scanned by the analyzer. The testing result of CYFRA21-1 will be calculated by the Biopanda Fluorescence Immunoassay Device to show PCT concentration in specimen.

REAGENTS

The test contains CYFRA21-1 antibody coated with cellulose nitrate membrane as the capture reagent and CYFRA21-1 antibody which conjugated with fluorescence particles as the detection reagent.

PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
3. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
4. Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
5. Do not interchange or mix reagents from different lots.
6. Humidity and temperature can adversely affect results.
7. Used testing materials should be discarded in accordance with local regulations.
8. Read the entire procedure carefully prior to any testing.
9. The Biopanda CYFRA21-1 FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) by approved medical professionals.

STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.
3. Do not freeze.
4. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS

- 25 x foil wrapped CYFRA21-1 Rapid Test Devices
- 25 x Specimen collection tubes with dilution buffer
- 1 x ID card (CYFRA21-1)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Device (BR-FIA-2000)

SPECIMEN COLLECTION AND PREPARATION

1. Before performing the test, please make sure that all components are brought to room temperature (15-30°C). Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.
2. Take a tube with buffer solution out of the kit. Document patients name or ID on it. Open the screw cap.

BLOOD SAMPLE COLLECTION

1. Collect the specimen according to standard procedures.
2. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8 °C for up to 1 day, for long term storage, specimens should be kept below -20 °C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by finger stick should be tested immediately.
3. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
4. EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

SAMPLE DILUTION / SAMPLE STABILITY

1. The specimen (20 µl of whole blood, serum, or plasma) can be added directly with the micro pipette into the buffer.
2. Close the tube and shake the sample by hand vigorously for approximately 10 seconds to mix the sample and dilution buffer.
3. Let the diluted sample rest for approximately 1 minute.
4. The sample can then be used immediately or stored at 2-8°C for up to 8 hours.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for the complete instructions on use of the Test. The test should be conducted at room temperature.

Allow the test, specimen, buffer and/or controls to reach room temperature (15-30°C) prior to testing.

1. Turn on the Analyser. Then according to the user requirement, select “Standard test” or “Quick test” mode.
2. Take out the ID card and insert it into the Analyser port.
3. **Whole Blood/Serum/plasma**: Transfer 20 µl of whole blood, serum, or plasma into the buffer tube, mix the specimen and the buffer thoroughly.
4. **Add diluted specimen with a Pipette**: Pipette 75 µl of diluted specimen into the sample well of the test Device. Start the timer at the same time.
5. There are two test modes for the Biopanda Fluorescence Immunoassay Device; Standard Test mode and Quick Test mode. Please refer to the user manual of the Biopanda Fluorescence Immunoassay Device for details.

“Quick test” mode: Insert the test device into the Analyser at 15 minutes after sample application and click "New Test", the Analyser will automatically give the test result after a few seconds.

“Standard test” mode: Insert the test device into the Analyser immediately

after sample application, click "New test" at the same time, the Analyser will automatically count down the 15 minutes. After the countdown, the Analyser will give the result at once.

INTERPRETATION OF RESULTS

The result of tests for CYFRA21-1 is calculated by the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) and displays the result on the screen. For additional information, please refer to the user manual of the Biopanda Fluorescence Immunoassay Device.

Linearity range of the Biopanda CYFRA21-1 FIA Rapid Test Device is 1.0-300 ng/ml.

QUALITY CONTROL

Each Biopanda CYFRA21-1 FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Device. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Device indicating that the test should be repeated. An invalid result from the internal control causes an "N/A" message on the Biopanda Fluorescence Immunoassay Device. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

1. The Biopanda CYFRA21-1 FIA Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the quantitative detection of CYFRA21-1.
2. The Biopanda PCT FIA Rapid Test Device will only indicate the presence of CYFRA21-1 antigen in the specimen and should not be used as the sole criterion for diagnosis.
3. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
5. Other factors interfering with the test and causing erroneous results include technical/ procedural errors, degradation of the test components as well as presence of interfering substances in the test samples.
6. The results of the Biopanda CYFRA21-1 FIA Tests are based on measuring the levels of CYFRA21-1 in a specimen. It should not be used as the sole criterion for treatment decisions. If the result is positive, other clinical findings and alternative test methods are recommended to reach proper medical treatments.

EXPECTED RESULTS

Concentrations	Clinical Reference
0-3.3 ng/ml	Normal
>3.3 ng/ml	High

Each laboratory should determine the applicability of the reference range through experiments, and establish its own reference value range if necessary to ensure that it can correctly reflect the situation of a particular population.

PERFORMANCE CHARACTERISTICS

1. **ACCURACY**: The test deviation is ≤±15%.
2. **SENSITIVITY**: The Biopanda CYFRA21-1 FIA Rapid Test Device can detect levels of CYFRA21-1 as low as 1.0 ng/ml in whole blood, serum or plasma.
3. **DETECTION RANGE**: 1.0-300 ng/ml
4. **LINEARITY RANGE**: 1.0-300 ng/ml, R≥0.990
5. **PRECISION**

C.V. is ≤ 15%.

INTRA-LOT PRECISION

Within-run precision has been determined by using 10 replicates of 2 specimens containing of CYFRA21-1. C.V. is ≤15%.

INTER-LOT PRECISION

Between-run precision has been determined by using 10 replicates for each of three lots using 2 specimens of CYFRA21-1. C.V. is ≤15%.

6. **METHOD COMPARISON**

The assay was compared with CYFRA21-1 Test commercially available with 105 samples. The correlation coefficient(r) is 0.990.

REFERENCES

1. Stieber P, Dienemann H, Hasholzner U, et al. Comparison of cytokeratin fragment 19 (CYFRA 21–1), tissue polypeptide antigen (TPA) and tissue polypeptide specifific antigen (TPS) as tumour markers in lung cancer. Eur J Clin Chem Clin Biochem 1993;31(10):689– 694.
2. Kawaguchi H, Ohno S,MiyazakiM, et al. CYFRA21–1 determination in patients with esophageal squamous cell carcinoma: Clinical utility for detection of recurrences. Cancer 2000;89(7):1413–1417.
3. Tomita M, Shimizu T, Ayabe T, Yonei A, Onitsuka T. Prognostic signifificance of tumour marker index based on preoperative CEA and CYFRA21–1 in non-small cell lung cancer. Anticancer Res 2010;30(7):3099–3102.
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6. Nakayama M, Satoh H, Ishikawa H, et al. Cytokeratin 19 fragments in patients with non-malignant respiratory diseases. Chest 2003;123(6):2001-2006.
7. Nakahama H, Tanaka Y, Fujita Y, et al. CYFRA21-1 and ProGRP, tumor markers of lung cancer are elevated in chronic renal failure patients. Respirology 1998;3:207-210.

Index of Symbols

	Manufacturer		Tests per kit		Do not reuse test
IVD	*In vitro* diagnostic medical device		Expiration date	REF	Catalogue number
	Store between 4-30°C	LOT	Lot Number		Consult instructions for use
	Do not use if package is damaged				

Thank you for purchasing Biopanda's CYFRA21-1 FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.

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