DRG

コスモ・バイオ株式会社

DRG® CYFRA 21-1 ELISA (EIA-3943)

CE

研究用

REVISED 14 MAR. 2005

1 INTRODUCTION

The **DRG® CYFRA 21-1 Enzyme Immunoassay Kit** provides materials for the quantitative determination of CYFRA 21-1 in serum and Heparin plasma.
This assay is intended for in vitro diagnostic use only.

CYFRA 21-1 is a fragment of cytokeratin 19. Although expressed in all body tissues its major occurrence is in the lung, particularly in lung cancer tissues. The major diagnostic importance of CYFRA 21-1 as a tumor marker is in differential diagnosis, prognosis, and aftercare of non-small-cell lung cancer (NSCLC) patients. Additionally, CYFRA 21-1 has been described as a tumor marker for the monitoring of bladder cancer.

The DRG® CYFRA 21-1 ELISA uses the two mouse monoclonal antibodies KS19.1 and BM19.21 to determine cytokeratin 19 fragments.

2 PRINCIPLE OF THE TEST

The DRG® CYFRA 21-1 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a CYFRA 21-1 molecule.
An aliquot of patient sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti- CYFRA 21-1 monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of CYFRA 21-1 in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CYFRA 21-1 in the patient sample.

3 PRECAUTIONS

- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.

REVISED 14 MAR. 2005

- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH.
- The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 KIT COMPONENTS

4.1 Contents of the Kit

1. **Microtiter wells**, 12x8 (break apart) strips, 96 wells
   Wells coated with monoclonal anti-CYFRA 21-1 antibody
2. **Standard (Standard 0-4)**, 5 vials (lyophilized), 1.0 ml
   Concentrations: 0; 3; 10; 25; 50 ng/ml
   see „Preparation of Reagents“;
   contain 0.3% Proclin as a preservative.
3. **Control**, 1 vial (lyophilized), 1.0 ml,
   see „Reagent Preparation“
   Control values and ranges please refer to vial label or QC-Datasheet
   contains 0.3% Proclin as a preservative
4. **Sample Diluent**, 1 vial, 3 ml, ready to use,
   contains 0.3% Proclin as a preservative
5. **Enzyme Conjugate 21X concentrate**, 1 vial, 0.5 ml,
   anti-CYFRA 21-1 antibody conjugated to horseradish peroxidase
   see „Preparation of Reagents“
   contains 0.3% Proclin as a preservative
6. **Conjugate Diluent**, 1 vial, 7 ml, ready to use
   contains 0.3% Proclin as a preservative
7. **Substrate Solution**, 1 vial, 14 ml, ready to use,
   TMB
8. **Stop Solution**, 1 vial, 14 ml, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **Wash Solution**, 1 vial, 30 ml (40X concentrated),
   see „Preparation of Reagents“

Note: Additional *Sample Diluent* for sample dilution is available on request.

4.1.1 Equipment and material required but not provided

– A microtiter plate calibrated reader (450±10 nm)(e.g. the DRG Instruments Microtiter Plate Reader).
– Calibrated variable precision micropipettes.
– Absorbent paper.
– Aqua dest.

4.2 Storage and stability of the Kit

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Note: *The reconstituted standards and control are stable for at least 4 weeks at 2-8°C. For longer storage freeze at -20°C.*

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 1 ml Aqua dest.

Note: The reconstituted standards are stable for at least 4 weeks at 2-8°C. For longer storage freeze at -20°C.

Control

Reconstitute the lyophilized content with 1 ml Aqua dest. and let stand for 10 minutes in minimum. Mix the control several times before use.

Note: The reconstituted control is stable for at least 4 weeks at 2-8°C. For longer storage freeze at -20°C.

Wash Solution

Dilute 30 ml of concentrated Wash Solution with 1170 ml deionized water to a final volume of 1200 ml.

The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate
Dilute Enzyme Conjugate concentrate 1:21in Conjugate Diluent.
Stability of the prepared Enzyme-Conjugate: Use within 24 hours.

Example:
If the whole plate is used, dilute 300 µl Enzyme conjugate (21x conc.) with 6 ml Conjugate Diluent to a total volume of 6.3 ml.
If the whole plate is not used at once prepare the required quantity of Enzyme Conjugate by mixing 25 µL of Enzyme Conjugate 21X conc. with 0.5 mL of Conjugate Diluent per strip (see table below):

No. of strips	Enzyme Conjugate 21X conc. (µl)	Conjugate Diluent (ml)
1	25	0.5
2	50	1.0
3	75	1.5
4	100	2.0
5	125	2.5
6	150	3.0
7	175	3.5
8	200	4.0
9	225	4.5
10	250	5.0
11	275	5.5
12	300	6.0

4.4 Disposal of the Kit
The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits
In case of any severe damage of the test kit or components, DRG® have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

REVISED 14 MAR. 2005

5 SPECIMEN

Serum or Heparin plasma can be used in this assay.
Citrate plasma results in decreased, EDTA in strongly increased values.
Do not use haemolytic, icteric or lipaemic specimens.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occured. Patients receiving anticoagulant therapy may require increased clotting time.
Plasma:
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001.)

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 2days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µl Serum + 90 µl Sample Diluent (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Sample Diluent (mix thoroughly).

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same. Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **50 µl** of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense **50 µl** Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **30 minutes** at room temperature without covering the plate.
6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (350 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µl** of Substrate Solution to each well.
8. Incubate for **30 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µl** of Stop Solution to each well.
10. Read the OD at **450±10 nm** with a microtiter plate reader **within 10 minutes** after adding the Stop Solution.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a standard curve with the CYFRA 21-1 ELISA.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/ml)	0.05
Standard 1 (3 ng/ml)	0.23
Standard 2 (10 ng/ml)	0.63
Standard 3 (25 ng/ml)	1.37
Standard 4 (50 ng/ml)	2.35

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG® CYFRA 21-1 ELISA the following values are observed:

Population	Valid N	5% Percentile	95% Percentile
Men and women	71	0.37 ng/ml	2.82 ng/ml

8 ASSAY CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 0 – 50 ng/ml.

8.2 Specificity of Antibodies (Cross Reactivity)

Sera of healthy individuals did not react with the DRG® CYFRA 21-1 ELISA

8.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be < 0.266 ng/ml.

8.4 Precision

8.4.1 Intra Assay Variation

The within assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	5.64	1.9
2	20	7.33	1.9
3	20	23.21	2.3

8.4.2 Inter Assay Variation

The between assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	11	5.54	7.6
2	11	8.01	4.8
3	11	13.93	7.4

8.5 Accuracy

8.5.1 Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG® directly.

8.5.2 Recovery

Samples have been spiked by adding CYFRA 21-1 with known concentrations
The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Added Concentration (ng/ml)	Measured Conc. (ng/ml)	Expected Conc. (ng/ml)	Recovery (%)
1	0.0	5.33		
	2.0	6.95	7.33	94.8
	4.0	9.20	9.33	98.6
	8.0	12.89	13.33	96.7
	16.0	21.27	21.33	99.7
2	0.0	7.75		
	2.0	9.79	9.75	100.4
	4.0	11.89	11.75	101.2
	8.0	14.75	15.75	93.7
	16.0	23.15	23.75	97.5
3	0.0	13.39		
	2.0	14.91	15.39	96.9
	4.0	17.05	17.39	98.0
	8.0	19.57	21.39	91.5
	16.0	28.51	29.39	97.0

8.5.3 Linearity

Sample	Dilution	Measured Conc. (ng/ml)	Expected Conc. (ng/ml)	Recovery (%)
1	None	9.06	9.06	
	1:2	4.61	4.53	101.9
	1:4	2.21	2.27	97.6
	1:8	1.11	1.13	98.0
	1:16	0.52	0.57	91.8
2	None	15.33	15.33	
	1:2	7.56	7.67	98.6
	1:4	3.78	3.83	98.6
	1:8	1.71	1.92	89.2
	1:16	0.86	0.96	89.8
3	None	30.48	30.48	
	1:2	15.49	15.24	101.6
	1:4	7.15	7.62	93.8
	1:8	3.52	3.81	92.4
	1:16	1.82	1.91	95.6

9 LIMITATIONS OF USE

9.1 Interfering Substances

Any improper handling of samples or modification of this test might influence the results.
Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.
The assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.

9.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CYFRA 21-1 in a sample.

9.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 400ng/ml of CYFRA 21-1.

10 LEGAL ASPECTS

10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially

relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG®.

10.2 Therapeutical Consequences

Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 10.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutical consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutical consequences.

10.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 10.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11 REFERENCES

1. Petra Stieber CYFRA 21-1 (Cytokeratin-19-Fragment) in Lothar Thomas, Labor and Diagnose, TH Books, Frankfurt, Germany.
2. J-L Pujol, O Molinier, W Ebert, J-P Daures, F Barlesi, G Bucceri, M Paesmans, E Quoix, D Moro-Sibilot, M Szturmowicz, J-M Brechot, T Muley and J Grenier (2004) British Journal of Cancer 90(11):2097-2105.