DRG® CYFRA 21-1 CLIA (CLA-4731)

RUO in the USA

CE

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1 INTRODUCTION

1.1 Intended Use

The **DRG CYFRA 21-1 CLIA** is a chemiluminescence immunoassay for measurement of CYFRA 21-1 in serum and heparin plasma. In the United States, this kit is intended for Research Use Only.

1.2 Explanation

The DRG CYFRA 21-1 CLIA 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 CLIA is a **c**hemi**l**uminescence **i**mmuno**a**ssay (CLIA), based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on the CYFRA 21-1 molecule.

An aliquot of donor sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti CYFRA 21-1 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.

After addition of the substrate solution, the intensity of emitted light is proportional to the concentration of CYFRA 21-1 in the donor sample.

3 WARNINGS AND PRECAUTIONS

1. For professional use only.
2. All reagents of this 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.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the donor samples will not be affected.

10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate luminometer.
16. The luminescence substrate reagents (*Reagent A* and *Reagent B*) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
17. 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.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 strips, 96 wells (break apart);
   Wells coated with a anti-CYFRA 21-1 antibody (monoclonal).
2. ***Standard (Standard 0-4)***, 5 vials (lyophilized), 1.0 mL;
   Concentrations: 0 – 3 – 10 – 25 – 50 ng/mL
   see "*Reagent Preparation* ";
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
3. ***Control***, 1 vial (lyophilized), 1.0 mL;
   see "*Reagent Preparation* ".
   For control values and ranges please refer to vial label or QC-Datasheet.
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
4. ***Sample Diluent***, 1 vial, 3 mL, ready to use;
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
5. ***Enzyme Conjugate* 21X concentrate**, 1 vial, 0.5 mL,
   anti-CYFRA 21-1 antibody conjugated to horseradish peroxidase
   see "*Reagent Preparation* "
   * contains 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservatives.
6. ***Conjugate Diluent***, 1 vial, 7 mL, ready to use.
   * contain 0.03% Proclin 300, 0.015% BND and 0.010% MIT as preservative.
7. ***Substrate Solution***,
   Reagent A, 1 vial, 2.0 mL, *Note: light sensitive!*
   Reagent B, 1 vial, 2.0 mL, *Note: light sensitive!*
   Reagent C, 1 vial, 5.0 mL
   see "*Reagent Preparation*".
8. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „ *Reagent Preparation* ".

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

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

4.2 Materials required but not provided

- A microtiter plate luminometer.
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer

– Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

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

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

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

4.4 Reagent Preparation

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°C to 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°C to 8°C. For longer storage freeze at -20°C.

Wash Solution

Add deionized water to the 40X concentrated 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 1:21 in *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

Chemiluminescence Substrate Solution

Mix **1 part** of the chemiluminescence ***Reagent A*** with **1 part** of ***Reagent B*** and dilute this mixture 1:2 with ***Reagent C***. This gives the ready to use substrate solution.

The prepared substrate solution is stable for one hour. Prepare fresh before use.

If the whole plate is to be used prepare the substrate solution as follows:

Add 1.5 mL of each *Reagent A* and *Reagent B* into 3 mL *Reagent C*.

4.5 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).

4.6 Damaged Kits

In case of any severe damage to the kit or components, DRG has to be informed in writing, at the 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.

5 SPECIMEN COLLECTION AND PREPARATION

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.

Please note: Samples containing sodium azide should not be used in the assay.

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 occurred. Donors 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 and Preparation

Specimens should be capped and may be stored for up to 2 days at 2°C to 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 ASSAY 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.
- Light intensity 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 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **50 µL** freshly diluted ***Enzyme Conjugate*** (see "*Reagent Preparation*") into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted *Wash Solution* (300 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!
6. Add **50 µL** of the freshly prepared Substrate Solution to each well. *(See* "*Reagent Preparation*".*)*
7. Incubate for **10 minutes** at room temperature.
8. Read the RLU with a microtiter plate luminometer **within 20 minutes** after incubation time of substrate.

6.3 Calculation of Results

1. Calculate the average Relativ Light Units (RLU) values for each set of standards, controls and donor samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. Using the mean RLU 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. 4 Parameter Logistics is the preferred method. 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 or reported as > 50 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data** is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard [ng/mL]	RLU (x10^3)	RLU/RLU$_{max}$ [%]
Standard 0 (0)	8.7	0.3
Standard 1 (3)	447	16.9
Standard 2 (10)	1186	44.9
Standard 3 (25)	2012	76.1
Standard 4 (50)	2642	100.0

** It is recommended to use the RLU/RLU$_{max}$ values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show different RLU values, however, the RLU/RLU$_{max}$ values remain consistent.

7 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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 donor 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 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

8.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 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.

8.2 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 LEGAL ASPECTS

9.1 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.

9.2 Liability

Any modification of the kit and/or exchange or mixture of any components of different lots from one 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 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

10 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)
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