



DRG[®] CYR61 Elisa (EIA-5108)



Revised 16 Jan. 2012 rm (Vers. 3.1)

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTRODUCTION

1.1 Intended Use

The **DRG Cyr61 ELISA** is an enzyme immunoassay for measurement of Cyr61 in serum, plasma and urine.

For Research Use Only (RUO)

2 PRINCIPLE OF THE TEST

The DRG Cyr61 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site of the Cyr61 molecule.

An aliquot of specimen sample containing endogenous Cyr61 is incubated in the coated well with assay buffer and enzyme conjugate, which is a rabbit anti-Cyr61 polyclonal antibody.

After incubation, the unbound conjugate is washed off.

Finally, Enzyme Complex, which is a goat anti-rabbit antibody conjugated with horseradish peroxidase, is added, and after incubation, unbound enzyme complex is washed off.

The amount of bound peroxidase is proportional to the concentration of Cyr61 in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Cyr61 in the specimen sample.

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3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. 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.
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 specimen 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 readers.
16. 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.
17. Avoid contact with *Stop Solution* containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
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. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. 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.



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4 REAGENTS

4.1 Reagents provided

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells;
Wells coated with anti-Cyr61 antibody (monoclonal).
2. **Standard (Standard 0-5)**, 6 vials, lyophilized, 1 mL each;
Concentrations: 0; 40; 100; 250; 500; 1000 pg/mL
See „Preparation of Reagents“;
Contain non-mercury preservative.
3. **Control Low & High**, 2 vials, lyophilized, 1 mL each;
See „Preparation of Reagents“;
For control values and ranges please refer to vial label or QC-Datasheet.
Contain non-mercury preservative.
4. **Assay Buffer**, 1 vial, 7 mL, ready to use,
Contains non-mercury preservative.
5. **Enzyme Conjugate**, 1 vial, 3 mL, ready to use,
rabbit anti-CYR61 antibody;
Contains non-mercury preservative.
6. **Enzyme Complex**, 1 vial, 14 mL, ready to use,
goat anti-rabbit-HRP conjugate.
Contains non-mercury preservative.
7. **Substrate Solution**, 1 vial, 14 mL, ready to use,
Tetramethylbenzidine (TMB).
8. **Stop Solution**, 1 vial, 14 mL, ready to use,
contains 0.5 M H₂SO₄.
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 *Standard 0* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- 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.



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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.

Opened kits retain activity for six weeks if stored as described above.

4.4 Reagent Preparation

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

Standard

Reconstitute the lyophilized content of the vials with 1.0 mL deionized water and let stand for 10 minutes in minimum. Mix the vials several times before use.

Note: The reconstituted standards are stable for 1 week at 2 °C to 8 °C.

Control

Reconstitute the lyophilized content of the vials with 1.0 mL deionized water and let stand for 10 minutes in minimum. Mix the vials several times before use.

Note: The reconstituted controls are stable for 1 week at 2 °C to 8 °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.

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 Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test 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.



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5 SPECIMEN COLLECTION AND PREPARATION

Serum, plasma (EDTA or heparin plasma) and urine can be used in this assay.

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. Samples containing anticoagulant 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 EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001)

Urine:

First clean genital area with mild desinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container.

5.2 Specimen Storage and Preparation

Serum, Plasma:

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time (up to six months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Urine:

Directly after collection, the urine should be centrifuged (e.g. at 2,000 g) to remove cellular debris. Use supernatant for analyte quantification

The supernatant may be stored for up to 8 hours at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time should be frozen at -20 °C. Thawed supernatant should be inverted several times prior to testing.



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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 *Standard 0* 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 1:2: 50 μ L sample + 50 μ L *Standard 0* (mix thoroughly)
- b) dilution 1:5: 20 μ L sample + 80 μ L *Standard 0* (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.
- 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.



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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 **25 µL** of Assay Buffer in all wells.
3. Dispense **50 µL** of each *Standard, Control* and **samples** with new disposable tips into appropriate wells.
4. Dispense **25 µL Enzyme Conjugate** into each well.
5. Incubate for **120 minutes** at room temperature on a plate shaker with ~ 700 rpm.
6. Briskly shake out the contents of the wells.
Rinse the wells **4 times** with **400 µL** diluted *Wash Solution* (manual washing: 4 times with 300 µL diluted *Wash Solution*). 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. Dispense **100 µL Enzyme Complex** in all wells.
8. Incubate for **30 minutes** at room temperature on a plate shaker with ~ 700 rpm.
9. Briskly shake out the contents of the wells.
Rinse the wells **4 times** with **400 µL** diluted *Wash Solution* (manual washing: 4 times with 300 µL diluted *Wash Solution*). Strike the wells sharply on absorbent paper to remove residual droplets.
10. Add **100 µL of Substrate Solution** to each well.
11. Incubate for **15 minutes** at room temperature.
12. Stop the enzymatic reaction by adding **100 µL of Stop Solution** to each well.
13. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
It is recommended that the wells be read **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 specimen samples.
2. Using semi-logarithmic graph paper, 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. 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 > 1000 pg/mL. For the calculation of the concentrations this dilution factor has to be taken into account.



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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	Optical Units (450 nm)
Standard 0 (0 pg/mL)	0.09
Standard 1 (40 pg/mL)	0.17
Standard 2 (100 pg/mL)	0.27
Standard 3 (250 pg/mL)	0.57
Standard 4 (500 pg/mL)	1.12
Standard 5 (1000 pg/mL)	2.14

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.

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 LEGAL ASPECTS

8.1 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.

8.2 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.



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9 REFERENCES / LITERATURE

1. K. Katsube, K. Sakamoto, Y. Tamamura and A. Yamaguchi. Role of CCN, a vertebrate specific gene family, in development. *Develop. Growth Differ.* (2009) 51, 55–67.
2. A. Leask, and D. J. Abraham. All in the CCN family: essential extracellular signalling modulators emerge from the bunker. *Journal of Cell Science* (2006) 119, 4803-10.
3. W.C. Sin, J.F. Bechberger, W.J. Rushlow, and C.C. Naus. Dose-dependent differential upregulation of CCN1/Cyr61 and CCN3/NOV by the gap junction protein connexin43 in glioma cells. *J. of Cellular Biochemistry* (2008) 103, 1772–1782.
4. M.-T. Lin, C.-Y. Zuon, C.-C. Chang, S.-T. Chen, C.-P. Chen, B.-R. Lin, M.-Y. Wang, Y.-M. Jeng, K.-J. Chang, P.-H. Lee, W.-J. Chen, and M.-L. Kuo. Cyr61 induces gastric cancer cell motility/invasion via activation of the integrin/nuclear factor- κ B/cyclooxygenase-2 signaling pathway. *Clin. Cancer Res.* (2005) 11, 5809-5820.
5. Y. Chen and A.-Y. Du. Functional properties and intracellular signaling of CCN1/Cyr61. *J. Cellular Biochemistry* (2007) 100, 1337-1345.
6. D. Sampath, Y. Zhu, R.C. Winneker, Z. Zhang. Aberrant expression of Cyr61, a member of the CCN (CTGF/Cyr61/Cef10/NOVH) family, and dysregulation by 17 beta-estradiol and basic fibroblast growth factor in human uterine leiomyomas. *J. Clin. Endocrinol. Metab.* (2001a) 86, 1707–1715.
7. W.G. Jiang, G. Watkins, O. Fodstad, A. Douglas-Jones, K. Mokbel and R.E. Mansel. Differential expression of the CCN family members Cyr61, CTGF and Nov in human breast cancer. *Endocrine-Related Cancer* (2004) 11, 781–791.

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