



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,
Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 9059-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
GASTROINTESTINAL CANCER ANTIGEN (CA19-9)

CA-199

Cat # 9059-16

Enzyme Immunoassay for the Quantitative Measurement of Gastrointestinal Cancer Antigen (CA19-9) in Human Serum

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric

methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL1 and MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF CA19-9 IMMUNOASSAY

A group of mucin type glycoprotein Sialosyl Lewis Antigens (SLA), such as CA19-9 and CA19-5, have come to be recognized as circulating cancer associated antigens for gastrointestinal cancer.

CA19-9 represents the most important and basic carbohydrate tumor marker. The immunohistologic distribution of CA19-9 in tissues is consistent with the quantitative determination of higher CA19-9 concentrations in cancer than in normal or inflamed tissues. Recent reports indicate that the serum CA19-9 level is frequently elevated in the serum of subjects with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA, elevated CA19-9 is suggestive of gallbladder neoplasm in the setting of inflammatory gallbladder disease. This tumor-associated antigen may also be elevated in some non-malignant conditions. Research studies demonstrate that serum CA19-9 values may have utility in monitoring subjects with the above-mentioned diagnosed malignancies. It has been shown that a persistent elevation in serum CA19-9 value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising serum CA19-9 value may be associated with progressive malignant disease and poor therapeutic response. A declining CA19-9 value may be indicative of a favorable prognosis and good response to treatment.

TEST PRINCIPLE

The CA19-9 EIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The CA19-9 molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of CA19-9 antigen in the sample. By reference to a series of CA19-9 standards assayed in the same way, the concentration of CA19-9 in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

1. Murine monoclonal anti-CA19-9 coated 96 well microtiter plate.
2. Assay buffer; 12 ml.
3. Enzyme conjugate reagent, 12 ml.
4. CA19-9 reference standards (liquid, one set), containing 0, 15, 30, 60, 120, and 240 U/ml CA19-9, Ready for use.
5. 50x Wash Buffer Concentrate, 15ml

6. Chemiluminescence Reagent A, 6.0 ml
7. Chemiluminescence Reagent B, 6.0 ml

Materials required but not provided:

1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 0.2ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

REAGENT PREPARATION

1. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50** μ L of CA19-9 standards, specimens, and controls into appropriate wells.
2. Dispense **100** μ L of Assay Buffer to each well. Mix gently for 30 seconds.
3. Incubate at 37°C for 60 minutes.
4. Remove the incubation mixture by emptying the plate content into a waste container.
5. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
7. Dispense **100** μ L of enzyme conjugate reagent into each well. Mix well.
8. Incubate at 37°C for another 60 minutes.
9. Remove the contents and wash the plate as described in step 4, 5 and 6 above.
10. Dispense **100** μ L Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
11. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

CALCULATION OF RESULTS

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend using proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against CA19-9 concentration in Units/ml on linear graph paper, with RLU 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 of CA19-9 in Units/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

CA19-9 (Unit/ml)	Relative Light Units (RLU) (10 ⁵)
0	0.97
15	10.86
30	19.46
60	40.44
120	69.77
240	97.98

EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA19-9 assay values below 35 U/ml. The minimum detectable concentration of CA19-9 in this assay is estimated to be 5 U/ml.

REFERENCES

1. Glenn, J., Steinberg, W.M., Kurtzman, S.H., et al. Evaluation of the utility of a radioimmunoassay for serum CA 19-9 level in patients before and after treatment of carcinoma of the pancreas. **J. Clin. Oncol.** 1988; 6:462-8.
2. Hayakawa, T., Kondo, T., Shibata, T. et al. Sensitive serum markers for detecting pancreatic cancer. **Cancer** 1988; 61:1827-31.
3. Koprowski, H., Herly, M., Steplewski, Z., et al. Specific antigen in serum of patients with colon carcinoma. **Science** 1981; 212:53-5.
4. Malesci, A., Tommasini, M.A., Bonato, C. et al. Determination of CA19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. **Gastroenterology** 1987; 92:60-7.
5. Safi, F, Roscher, R., Bittner, R., et al. High sensitivity and specificity of CA 19-9 for pancreatic carcinoma in comparison to chronic pancreatitis. Serological and immunohistochemical findings. **Pancreas** 1987; 2:398-403.
6. Steinberg, W. The clinical utility of CA 19-9 tumor associated antigen. **American J. of Gastroenterology** 1990; 85:350-355.
7. Steinberg, W.M., Gelfand, R., Anderson, K.K., et al. Comparison of the sensitivity and specificity of the CA 19-9 and carcinoembryonic antigen assays in detecting cancer of the pancreas. **Gastroenterology** 1986; 90:343-9.
8. Takasaki, H., Uchida, E., Tempero, M.A., et al. Correlative study on expression of CA 19-9 and DU-Pan-2 in tumor tissue and in serum of pancreatic cancer patients. **Cancer Res.** 1988; 48:1435-8.
9. Tatsuta, M., Yamamura, H., Iishi H., et al. Values of CA19-9 in the serum, pure pancreatic juice and aspirated pancreatic material in the diagnosis of malignant pancreatic tumor. **Cancer** 1985; 56:2669-73.

10. Wang, T.H. Lin, J.W., Chen, D.S., et al. Noninvasive diagnosis of advanced pancreatic cancer by real-time ultrasonography, carcinoembryonic antigen, and carbohydrate antigen 19-9. *Pancreas* 1986; 1:219-23.
11. Strom BL, Maislin G, West SL, et al. Serum CEA and CA19-9: potential future diagnostic or screening tests for gallbladder cancer? *Int. J. Cancer* 1990; 45:821

Date Adopted:	Reference No.
2008-05-18	DA-CA-199-2009



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 02/16/09