



DRG[®] CA-242 (Pancreatic-colon-rectal) ELISA (EIA-4581)



Revised 21 Sept. 2011 rm (Vers. 2.1)

USA:RUO

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

NAME AND INTENDED USE

The DRG[®] Pancreatic & colon-rectal Cancer (CA-242) is a solid phase enzyme linked immunosorbent assay (ELISA). This test provides measurement of CA-242 antigen

PRINCIPLE OF THE ASSAY

Pancreatic & rectal CA-242 cancer assay is a solid phase enzyme-linked immunosorbent system employing plastic wells coated with streptavidin. The sample, standards and controls and biotinylated anti-CA-242 antibodies are allowed to incubate in the wells. During the incubation, specific cancer antigen (CA-242) is bound to CA-242 antibodies on the wells. Unbound CA-242 antigen is removed by washing the wells with buffer. Enzyme conjugate is added to each well. After the incubation, unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the CA-242 antigen present in the sample. Upon addition of the chromogen substrate, the intensity of color developed is proportional to the concentration of CA-242 antigen in the sample and may be quantified by use of a photometric well reader at 450 nm wavelength.

WARNING AND PRECAUTION

The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.

References contains human serum should be treated as potentially infectious. All human based products should be using appropriate precaution.

Handle all references standards and test samples as potentially infectious agents. The standards were found negative for the hepatitis B and HIV I/II, HCV and hepatitis B virus, or other infectious agents, these materials should be handled at Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers of Disease control/ National Institutes of health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.

Never pipet by mouth. Avoid contact with skin.

Materials Provided

1. Microwell Strips (96 wells): Streptavidin coated wells. 8 x 12 strips.
2. Biotinylated Capture Antibody Solution (6 mL)



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3. Enzyme Conjugate (6 mL): Anti-CA-242 antibodies conjugated to horseradish peroxidase.
4. Specimen Diluent (11 mL): or zero standards.
5. Reference Standards (0.5 mL each vial) calibrated to 5, 25, 50, 100, and 200 U/mL
6. Low and High Control (0.5 mL each).
7. TMB Solution (11 mL): Buffer solution containing peroxide and Tetramethylbenzidine.
8. Washing Buffer Concentrate (100X) (10 mL): Prepare working solution by adding 10 ml washing buffer concentrate into 990 mL distilled water.
9. Stop solution: 2N HCl.
10. Well holder; for securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader with wavelength at 450 nm.
2. Pipetor with tips for measuring 25 µL and 100 µL
3. Clean plastic washing bottle of 1000 mL capacity for use in washing microwells with working washing buffer during testing procedures.

REAGENT PREPARATION

Prepare the working washing buffer by adding the entire contents of the Wash Buffer Concentrate to 1000 mL distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.

STORAGE AND STABILITY

Store the kits at 2-8°C and keep micro-wells in a dry bag with desiccants. Unopened reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture and allow clotting. Separate the serum by centrifugation at room temperature. If sera cannot be immediately assayed, they may be stored at -20° C for at least six months. Avoid repeated freezing and thawing of samples. Specimens obviously contaminated with bacteria should not be in use. Specimens turbid with high lipid concentrations should be clarified prior to assay.

PREPARATION FOR ASSAY

Bring all reagents and samples to room temperature (24±3°C) and mix gently before beginning the test.



Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.

Use new disposable tips for each specimen.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 25 μ L of Sample diluent into well #1 as a blank, 25 μ L of standards, samples or controls into appropriated well. Add 50 μ L of biotinylated solution (blue color) to each well except blank well.
3. Dispense 50 μ L Enzyme Conjugate into each well except blank well.
4. Incubate for 60 minutes at room temperature.
5. Wash five times with the Washing Buffer (300 μ L/well/each rinse).
6. Dispense 100 μ L of TMB Solution into each well.
7. Incubate for 30 minutes at room temperature.
8. Stop reaction by adding 50 μ L of Stop Solution to each well.
9. Zero a microreader with the bank and measure the absorbance of each well at 450 nm.

PROCEDURAL NOTE

Wash the microwells and remove water thoroughly to get the best results.

Pipet all reagents and samples into bottom of the well. Vortex mixing or shaking of wells after sample and reagent pipetting is not required.

The appropriate number of wells should be secured in a holder and all reagent and sample caps should be removed prior to the start of testing. This will permit pipetting at equal intervals without interruption. A maximum of 30 samples should be assayed at one time in order to minimize error due to timing differences between specimens.

Absorbance is a function of the time and temperature of incubation. It is recommended to have reagents, samples and needed wells ready to ensure the equal time for each pipetting without interruption.

CALCULATION OF RESULTS

Plot the concentration (x) of each Reference Standard against its absorbance (y) on graph paper.

Obtain the CA 242 antigen value of specimen sample by referring to the Standard curve as followed (these data are for demonstration purposes only and must not be used in place of data generated for each assay):

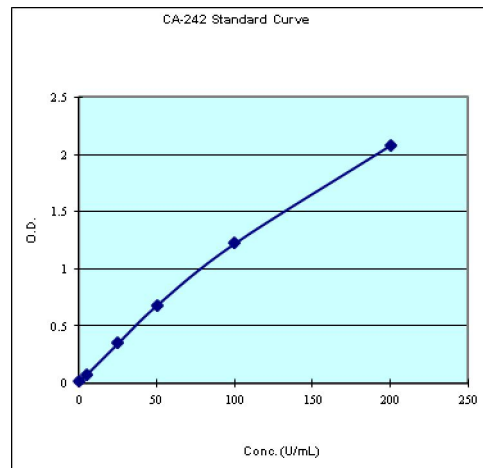
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Wells No.	Description (U/mL)	Absorbance (450 nm)	CA-242
A1		0.019	
A2	0	0.024	
B1		0.074	
B2	5	0.085	
C1		0.286	
C2	25	0.268	
D1		0.636	
D2	50	0.729	
E1		1.149	
E2	100	1.303	
F1		2.084	
F2	200	2.085	
G1		0.268	
G2	Sample 1	0.219	18.3
H1		1.108	
H2	Sample 2	1.065	94.5



It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its local environmental factors such as diet, climate, etc.



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APPLICATIONS & LIMITATIONS OF THE PROCEDURE

The DRG CA-242 Assay should not be used in cancer screening and should not replace any established clinical examination.

QUALITY CONTROL

Each laboratory should utilize internal controls at several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available source.

MINIMAL DETECTABLE CONCENTRATION

The detectable limit of CA-242 ELISA assay is 1 U/mL. The minimal detectable concentration of CA-242 is defined as that of CA 242 which corresponds to the absorbance that is two standard deviation from the mean absorbance of 10 replicate determinations of the sample diluent (0/mL).

REFERENCES

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