CA19-9

Enzyme immunoassay for the quantitative determination of CA19-9 in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV063 (96 Determinations)
1. INTRODUCTION

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 immunohistological 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 favourable prognosis and good response to treatment.

2. INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of CA19-9 in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells are precoated with monoclonal antibodies against CA19-9. During first incubation CA19-9 in samples and standards bind to the immobilised antibodies on the surface of the microtiter wells. Unbound substances will be removed by a subsequent washing step. During second incubation specific anti-IgG antibodies conjugated with peroxidase bind to CA19-9. Unbound conjugate will be removed by a subsequent washing step. In a third incubation the enzyme substrate TMB will be oxidized resulting in a blue colour. Addition of sulphuric acid (stop solution) stops the enzymatic reaction and turns the blue reaction product into yellow. Absorbance is measured at 450 nm. The amount of oxidized TMB is proportional to the amount of CA19-9 in the sample.

4. MATERIALS

4.1. Reagents supplied
- Coated Microplate: 12 breakapart 8-well snap-off strips coated with monoclonal antibodies against CA19-9; in aluminium foil.
- Assay buffer: 1 bottle containing 12 ml phosphate buffer (50mM, pH 7.4), 1 g/l BSA.
- Conjugate: 1 bottle containing 12 ml of horseradish peroxidase labelled anti-mouse CA19-9 antibodies.
- TMB Substrate Solution: 1 bottle containing 12 ml 3, 3’, 5, 5’-tetramethylbenzidine (H₂O₂-TMB 0.25 g/l) (avoid any skin contact).
- Wash solution 20x conc.: 1 bottle containing 30 ml (NaCl 9 g/l, Tween 20 0.05 g/l).
- Stop Solution: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- Standards: 6 bottles containing 1 ml standard solution with the following concentration:
  - Standard 0: 0 U/ml
  - Standard 1: 15 U/ml
  - Standard 2: 30 U/ml
  - Standard 3: 60 U/ml
  - Standard 4: 120 U/ml
  - Standard 5: 240 U/ml

4.2. Materials supplied
- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed
- 37 °C incubator
- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver a volume of 100 µl
- Distilled water
- Timer

5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.
6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (20…25°C) before starting the test run!

6.1. Coated microplate

The ready to use break apart snap-off strips are coated with monoclonal IgG antibodies against CA19-9. Store at 2…8 °C. Open the bag only when it is at room temperature. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C. Do not remove the adhesive sheets on the unused strips.

6.2. Conjugate

The conjugate is ready to use.

6.3. CA19-9 Standards

- The standards are ready to use. After first opening the standards are stable for another 6 months if stored at +4°C.

6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2…8°C in the dark. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2…8°C.

6.6 Wash Solution

Dilute the concentrated wash solution to 600 ml distilled or deionised water.

7. SPECIMEN COLLECTION AND PREPARATION

The CA19-9 assay can be performed in both serum and plasma. Samples can be stored at +2 to +8 °C for max. 5 days. For longer storage the specimen should be frozen. Avoid repeated freezing and thawing.

For samples with concentration higher than 240 U/ml dilute one part of sample with three parts of Standard 0.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 100 µl standards and samples into their respective wells.
2. Dispense 100 µl assay buffer in wells with standards and samples, not into the blank well. Cover with a foil.
3. Incubate for 60 min at 37 °C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well five times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Add 100 µl Conjugate to each well except blank.
6. Cover wells with the foil supplied in the kit.
7. Incubate for 60 min at 37°C.
8. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well five times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

9. Dispense 100 µl TMB Substrate Solution into all wells.

10. **Incubate for exactly 15 min at room temperature (+22…+28°C) in the dark.**

11. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.

12. Measure the absorbance of the specimen at 450 nm within 30 min after addition of stop solution against blank.

### 9. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of CA 19-9 for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

### 10. RESULTS

#### 10.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic). Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in U/ml.

#### 10.2. Reference values

Healthy women are expected to have CA19-9 values below 35 U/mL.

### 11. SPECIFIC PERFORMANCE CHARACTERISTICS

#### 11.1. Sensitivity

The lowest detectable concentration of CA 19-9 that can be distinguished from the zero standard is 5 U/ml at the 95% confidence limit.

#### 11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Concentration</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 15-3</td>
<td>1,000 U/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>CA 125</td>
<td>1,000 U/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>PSA</td>
<td>1,000 ng/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>PAP</td>
<td>1,000 ng/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>AFP</td>
<td>10,000 ng/ml</td>
<td>0.00</td>
</tr>
<tr>
<td>CEA</td>
<td>1,000 ng/ml</td>
<td>0.00</td>
</tr>
</tbody>
</table>

#### 11.3. Precision

**Intra Assay Variation**

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6%.

**Inter Assay Variation**

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 8.1%.

#### 11.4. Hook effect

The CA 19-9 ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 10,000 U/ml.
12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- The conc. Wash solution contains Proclin 300® as preservative.
- Standards and conjugate contain Gentamycin as stabilizer.
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- This method allows the determination of CA19-9 from 0 – 240 U/ml.
- For samples with concentration higher than 240 U/ml dilute one part of sample with three parts of Standard 0.

**WARNING:** Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE


15. ORDERING INFORMATION

Prod. No.: DNOV063 CA19-9 Determination (96 Determinations)
SCHEME OF THE ASSAY
CA19-9

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard 0 - 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard 0 - 5</strong></td>
<td>-</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td>-</td>
<td>-</td>
<td>100 µl</td>
</tr>
<tr>
<td><strong>Assay buffer</strong></td>
<td>-</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit
**Incubate for 60 min at 37 °C**
Wash each well **five times** with **300 µl** diluted Wash Solution

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<th></th>
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<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diluted conjugate</strong></td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit
**Incubate for 60 min at 37 °C**
Wash each well **five times** with **300 µl** diluted Wash Solution

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<th>Standard 0 - 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TMB</strong></td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

**Incubate for exactly 15 min at room temperature in the dark**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard 0 - 5</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stop solution</strong></td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Photometric measurement at 450 nm

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