Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

CE

# AccuDiag™ CA-12-5 ELISA Kit

**REF** 6502-16

IVD See External Label 2°C 8°C Σ 96 Tests

| CA-12-5 ELISA | |
|---|---|
| Method | Enzyme Linked Immunosorbent Assay |
| Principle | Sandwich Complex |
| Detection Range | 0-400 U/mL |
| Sample | 50 µL serum |
| Sensitivity | 5 U/ml |
| Incubation Time | 200 minutes |
| Shelf Life | 12 Months from the manufacturing date |

## PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

Enzyme Immunoassay for the Quantitative Measurement of Ovarian Cancer Antigen (CA-12-5) in Human Serum

## INTENDED USE

The CA-12-5 ELISA test is primarily intended for use as a monitoring and screening test. An abnormal result (i.e., elevated serum CA-12-5 level) can indicate ovarian cancer and suggests the need for further clinical management. The serum CA-12-5 test appears to be a useful tumor marker for patients in clinical remission, following treatment. Post-operative serum CA-12-5 values that fail to return to normal, strongly suggest the presence of a residual tumor. Tumor recurrence is often accompanied by a rise of serum CA-12-5 before progressive disease is clinically evident.

## SIGNIFICANCE AND SUMMARY

One in every 70 American women will develop ovarian cancer in her life. There are approximately 20,000 new cases of ovarian cancer diagnosed every year and more than 12,000 women die each year because of it. Ovarian cancer is the most malignant type of gynecological cancers, with an overall 5-year survival rate of only 30%. This is because diagnosis is often not made until the advanced stage. Cancer Antigen 125 (CA-12-5) is a surface antigen associated with epithelial ovarian cancer. In serum, CA-12-5 is associated with a high molecular weight glycoprotein. Serum concentrations of this tumor marker can be detected and measured by a murine monoclonal antibody. Published studies have indicated that elevated serum CA-12-5 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma. Serum CA-12-5 levels higher than normal can also be found in individuals with adenocarcinoma of the fallopian tube endometrium, certain non-gynecologic malignancies and some non-malignant conditions.

The serum CA-12-5 concentration is greater than 35 units per ml in about 60% of women with ovarian cancer. More than 80% of patients with disseminated ovarian cancer have serum CA-12-5 concentrations greater than 35 units per ml. The serum CA-12-5 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serum levels of CA-12-5 greater than 35 units per ml combined with pelvic examination increases the test specificity. Serial determinations of serum CA-12-5 further enhance the positive predictive value of the test for ovarian cancer. Serum CA-12-5 concentration may be useful in monitoring patients with diagnosed ovarian cancer. A persistently high serum CA-12-5 may be associated with progressive malignant disease and poor therapeutic response. On the other hand, a declining CA-12-5 value appears to be indicative of a favorable prognosis and a good response to treatment. Residual disease is confirmed in 95% of patients with serum CA-12-5 concentrations greater than 35 units per ml; however, negative results do not necessarily exclude the disease. To date, CA-12-5 is the most sensitive marker for residual epithelial ovarian cancer. CA-12-5 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

## ASSAY PRINCIPLE

The CA-12-5 Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti-CA-125 antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-CA-12-5 antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the CA-12-5 antibody coated microtiter wells. Then CA-12-5 antibody labeled with horseradish peroxidase (conjugate) is added. If human CA-12-5 is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the CA-12-5 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 3 hour incubation at 37°C, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of CA-12-5 is directly proportional to the color intensity of the test sample.

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at -20°C. Thawed samples must be mixed prior to testing.

## MATERIALS AND COMPONENTS

**Materials provided with the test kit**

1. Monoclonal anti-CA-12-5 –antibody coated microtiter plate 96 wells
2. Enzyme conjugate reagent 12 ml
3. TMB Substrate 12 ml
4. Stop Solution 12 ml
5. CA-12-5 reference standards, containing 0, 15, 50, 100, 200, and 400 Unit/ml of CA-12-5, in liquid form (ready to use) or lyophilized form
6. Wash Buffer Concentrate(50X) 15 ml
7. Control set (optional)

**Materials required but not provided**

1. Precision pipettes: 40µl-200µl, 200-1000µl
2. Disposable pipette tips
3. Distilled water
4. Vortex mixer
5. Absorbent paper or paper towel
6. Microtiter plate reader
7. Graph paper

## REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer Concentrate (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.

1. Secure the desired number of coated wells in the holder. Dispense **50**µl of CA-12-5 standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
2. Dispense **100**µl of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have a complete mixing in this step. Incubate at 37°C for 3 hours.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense **100**µl of TMB substrate into each well. Gently mix for 10 seconds. Incubate at room temperature for 20 minutes.
5. Stop the reaction by adding 100µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

**Important Notes**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

## RESULTS

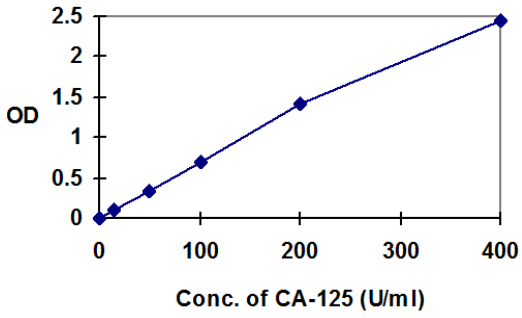
Calculate the mean absorbance value for each set of CA-12-5 reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CA-12-5 in units per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

## EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against CA-12-5 concentrations shown in the X axis.

| CA-12-5 Values (U/ml) | Absorbance (450nm) |
|---|---|
| 0 | 0.010 |
| 15 | 0.105 |
| 50 | 0.347 |
| 100 | 0.703 |
| 200 | 1.411 |
| 400 | 2.437 |

This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.



## EXPECTED VALUES AND SENSITIVITY

Healthy women are expected to have CA-12-5 assay values below 35 U/ml. The minimum detectable concentration of CA-12-5 in this assay is estimated to be 5 U/ml.

## LIMITATIONS OF THE PROCEDURE

There are some limitations of the assay:

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

## STORAGE

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.

1. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
2. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

## REFERENCES

1. Kenemans P, Yedema CA, Bon GG, von Mensdorff-Pouilly S. Ca125 in gynecological pathology a review. **Eur J Obstet Gynecol** 1993;49:115:124.
2. Saksela F. Prognostic markers in epithelial ovarian cancer. **Intl J Gynecol Pathol** 1993;12:156-161.
3. Farghaly SA. Tumor markers in gynecologic cancer. **Gynecol & Obstet Invest** 1992;34:65-72.
4. Welander CE. What do CA 125 and other antigens tell us about ovarian cancer biology. **Acta Obstet Gynecol Scand Sup** 1992;155:85-93.
5. McGowan L. Pathology of the ovary. **Curr Opin on Obstet Gynecol** 1991;3:580-586.
6. Niloff JM. Ovarian malignancy. **Curr Opin on Obstet Gynecol** 1991;3:66-72.
7. Olt G, Berchuck A, Bast RC. The role of tumor markers in gynecologic oncology. **Obstet Gynecol Survey** 1990;45-:570-577.
8. Diez M, Cerdan FJ, Ortega MD, Torres A, Picardo A, Balibrea JL. Evaluation of serum CA-12-5 as a tumor marker in non-small cell lung cancer. **Cancer** 1991;67:150-154.
9. Niloff JM, Klug TL, Schaetzl E. Elevation of serum CA-12-5 in carcinomas of the fallopian tube, endometrium, and endocervix. **AM J.Obstet Gynecol** 1984; 148:1057.

## MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

**Diagnostic Automation/Cortez Diagnostics, Inc.**
**21250 Califa Street, Suite 102 and 116,**
**Woodland Hills, California 91367 USA**

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| **Date Adopted** | **2022-09** |
| **REF 6502-16** | **AccuDiag™ - CA-12-5 ELISA** |
| **EC REP** | **CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** www.cepartner4u.eu |
| **Revision Date: 2017-09** | |