



## OncoEIA CA125

### Enzyme Immunoassay for Quantitative Determination of Cancer Antigen CA -125 In Human Serum

#### INTENDED USE

OncoEIA125ket is intended for the quantitative determination of cancer antigen CA125 in human serum.

CA125 is an oncofetal protein. It is a mucopolysaccharide with a molecular weight of over 200,000 Da. Normally in adult CA 125 exists in two forms. Free and membrane-bound. Membrane-bound antigen can be identified on the surface of epithelial cells of fallopian tubes, cervix, endometrium, bronchi, mammary gland and sudoriferous gland. High concentrations of free antigen can be detected in seminal liquid, breast milk, vaginal secretions, saliva, bronchoalveolar and intraperitoneal fluids. In bloodstream CA 125 persists in low concentrations. Increased concentration of serum CA 125 is a sign of ovarian pathology (either benign or malignant).

Quantitative determination of serum CA 125 is used for monitoring of patients with ovarian cancer for estimation of residual tumor. Diagnostic value of this method depends on histological type of tumor. It is the highest in the case of serous ovarian carcinomas in comparison with other types of carcinoma (e.g. mucinous carcinoma).

#### PRINCIPLE OF THE TEST

OncoEIA-CA 125 is a sandwich type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of CA 125 molecule. One of these antibodies is conjugated with horseradish peroxidase; the other is immobilized on the inner surface of microwells. CA 125-peroxidase conjugate. Then the wells are washed with wash buffer to remove any material not coloring appears. The color intensity is in direct proportion to the CA 125 concentration in sample. The enzyme reaction is stopped by dispensing the acidic solution (1N HCl) into the wells. Optical density of the solution in the wells is directly proportional to the CA 125 concentration in the samples. The standard curve is plotted by using the CA 125 concentration in the calibrators (x-axis) and their corresponding OD values (y-axis). The CA 125 concentration of the specimen is directly read off from the standard curve.

#### PATIENTS SAMPLES

##### Specimen collection and storage:

Blood is taken aseptically by venipuncture. After clotting, the serum is separated by centrifugation. Do not use plasma, hemolysed or lipemic serum and serum with sodium azide added as preservative.

Store serum samples at +2-+8°C for no more than 2 days; for longer storage it is recommended to aliquot and freeze at -20°C or below. Avoid repeated freezing.

##### Preparation before use:

Prior to assay, allow the samples to reach room temperature. Take care to agitate serum samples gently in order to ensure homogeneity.

If the expected CA 125 concentrations in the samples are higher than in **calibrator 5**, the samples should be diluted 30-fold and 900-fold with **Sample Diluent (D)** as follows:

Sample 1 (30-fold dilution): 290ul of Sample Diluent + 10ul of serum sample, mix thoroughly.

Sample 2 (900-fold dilution): 290ul of Sample Diluent + 10ul of serum sample 1, mix thoroughly.

Do not store diluted samples.

**Note: Samples must be diluted only with Sample Diluent included in the kit. Never use Sample Diluents from other kits.**

## 2.1. Kit Contents:

- microtiter plate : 12 breakable strips of 8 wells each (total 96 wells), coated with anti-CA 125 monoclonal antibodies 1 bag

- Sample diluent

TMB Substrate : 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide

CA 125 calibrators (protein-based buffer containing known CA 125 concentrations). For exact CA 125 Concentrations, see vial labels

- Conjugate . Contains anti-CA 125 antibodies conjugated with HRP

- wash buffer, 20X

- tetramethylbenzidine substrate solution, labeled "TMB solution", 1 vial, 14 ml;

- "Stop reagent"

- CA 125 control (protein-based buffer containing known CA 125 concentration), see vial labels

## 5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

- digital variable pipettes that cover volume ranges from 0.005 to 5 ml; with appropriate disposable tips;

- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;

- microplate shaker-thermostat, able to maintain temperature +37°C and shaking speed 500 to 800 rpm;;

- automatic microplate reader;

- volumetric cylinder;

- volumetric beaker;
- distilled water.

## **Size and storage**

**OncoCA 125** kit is designed for 96 determination. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 TMB substrate control in duplicates, provided that all the strips are used simultaneously.

Please take into consideration that calibrators should be measured in each separate assay.

It is also recommended to measure CA 125 concentration in the control each time. The number of separate experiments that can be performed with one kit (4 experiments) is therefore limited by the volume of calibrators.

NOTE: if used partially, kit should be utilized within a month after opening.

The expiry date of the kit is reported on the box label, expiry date for each component is indicated on the respective label.

Upon receipt, OncoEIA-CA 125 kit should be stored at 2-8, preferably in the original kit box.

If used for separate experiments, kit content should be stored as follows:

- the unused strips: in a firmly closed plastic bag at 2-8 until expiry date.
- opened vials with conjugate, sample diluent and TMB substrate solution at 2-8 for no more than 1 month.
- concentrated wash buffer : at 2-8 until expiry date.
- wash buffer prepared for use, in a firmly closed bottle for no more than 5 days at room temperature.
- reconstituted calibrators and control: at 2-8 for no more than 1 month after reconstitution.
- ready to use calibrators and control: at 2-8 for no more than 1 month after opening.
- stop reagent at 2-8 until expiry date.

## **Preparation before use**

Before the assay, allow all the kit components to reach a room temperature and stir thoroughly.

A. the microtitration plate consists of a frame and breakable strips, packed in the foil bag. Before opening, keep the bag at room temperature(18-25) for at least 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in the original plastic bag with ziplock closure and close firmly. Keep at 2-8 until expiry date stated on the label.

B. prepare the necessary volume of wash buffer by dilution the concentrate 20 fold with distilled or deionized water.

Mix thoroughly, avoiding foaming. Keep the prepared wash buffer firmly closed. Store at room temperature (18-25) for no more than 5 days.

The rest of the concentrated wash buffer should be stored firmly closed at 2-8 until expiry date.

C. calibrators and controls:

Lyophilized Calibrators and Control: gently tap on the vial caps to shake off all the dry matter.

Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5ml of distilled water to vials with lyophilized Calibrators and Controls, close each vial with the corresponding cap and leave for 10 min at room temperature (+18-+25), then stir gently, avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature with periodical gentle stirring. Make sure than no dry matter is left on the caps and walls of the vials. Store at +2-+8C for no more than 1 month.

E. Protect the **TMB Substrate solution** from direct light .

## 7. ASSAY PROCEDURE

**All the samples should be tested in duplicates.**

1. Bring all the reagents to room temperature before use. Stir gently, without causing foaming

2. Dispense **150** µl of conjugate into each well, **except A1-A2 wells.**

3. Dispense

**50** µl of CA 125 **Clibrators (0 to 5);**

**50** µl of CA 125 **Control (C);**

**50** µl of patients samples

Into the respective wells

Note: Total time of dispensing must not exceed 15 minutes, otherwise the result may be unreliable, because the time of incubation with conjugate will substantially vary for different samples.

4. Incubate strips for 2 hours while shaking (500-800rpm) at room temperature (18-25C).

5. Decant, then wash each well 5 times with 300 µl of wash buffer (prepared from B). Make sure that after the last washing cycle the residual buffer is thoroughly aspirated from the wells.

6. Immediately add 100 µl of TMB Substrate solution (F) into each well.

7. Incubate for 15-30 minutes at room temperature in the dark, depending on the color intensity.

8. Add 100 µl of stop solution reagent (G) to all the well and shake well for 1-2 minutes.

9. Read the optical density at a wavelength of 450 nm.

#### DATA PROCESSING

Data processing is done by a computer-assisted analysis by plotting the mean OD values of the calibrators at 450 nm versus their respective CA 125 concentrations using 4PL or 5PL fit (see typical standard curve) . Mean OD of the A1 –A2 wells is used as blank.

Any extrapolation of the standard curve to CA 125 concentration above the nominal value of Calibrator 5 (approximately 500 U/ml) is not permitted. In this case the sample should be additionally diluted 30-fold or 900-fold and re-tested.

#### TYPICAL EXAMPLE OF STANDARD CURVE

Do not use for evaluation of real assay data

#### REFERENCE VALUES

The range of CA 125 concentration up to 35U/ml was classified as normal . CA 125 concentration is above normal in 50% of women with primary ovarian cancer and in 80% of women with metastatic ovarian cancer. These limits should be considered as guidelines only.

It is highly recommended for each laboratory to determine its own reference range of CA 125 concentrations.

#### PERFORMANCE CHARACTERISTICS OF THE ASSAY

sample	dilution	Measured concentration, U/ml	Expected concentration, U/ml	Observed/expected concentration ratio, %
1	Undiluted	1057.8		
	1:4	214.02	264.45	81%
	1:8	113.52	132.22	86%
	1:16	58.218	66.11	88%
	1:32	32.37	33.06	98%
2	Undiluted	6597.3		
	1:10	682.76	659.73	103%
	1:20	309.6	329.86	94%
	1:40	153.13	164.93	93%
	1:80	86.761	82.47	105%
3	Undiluted	15313		
	1:50	337.43	306.26	110%
	1:100	157.47	153.13	103%
	1:200	82.745	76.56	108%%
	1:400	41.056	38.28	107%

#### Specificity:

No cross-reaction was detected between anti-CA125 monoclonal antibodies used in the assay and CWA, CA 19-9, CA 15-3 and CA 72-4 cancer antigens.

For OncoEIA-CA 125 kit high dose hook effect was not detected for concentrations up to 30000U/ml.

#### Analytical sensitivity(lower detection limit):

Analytical sensitivity of OncoEIA-CA 125 assay, or the lowest detectable concentration that can be distinguished from zero calibrator is 5 U/ml. it is defined as mean OD of 10 replicates of calibrator 0 plus two standard deviations.

#### LIMITATION OF THE METHOD

Any clinical diagnosis should not be based in the results of in vitro diagnostic methods alone. To state a diagnosis, the physician is supposed to consider all they available clinical and laboratory findings.

Assay stage and reagents used									
Conjugate,	-	150	150	150	150	150	150	150	150
Calibrator 0		50	-	-	-	-	--	-	-
Calibrator № 1, µl	-	-	50	-	-	-	-	-	-
Calibrator № 2, µl	-	-	-	50	-	-	-	-	-
Calibrator № 3, µl	-	-	-	-	50	-	-	-	-
Calibrator № 4, µl	-	-	-	-	-	50	-	-	-
Calibrator № 5, µl	-	-	-	-	-	-	50	-	-

Control, µl	–	–	–	–	–	–	–	50	–
Unknown sample, µl	–	–	–	–	–	–	–	–	50
Incubation №1	<b>2 hour on shaker-thermostat at room temperature</b>								
Wash (five times): wash buffer, µl	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300
TMB solution, µl	100	100	100	100	100	100	100	100	100
Incubation №2	<b>15-30 minutes in the dark at room temperature</b>								
Stop-reagent, µl	100	100	100	100	100	100	100	100	100
Stirring	<b>1-2 minutes on shaker</b>								
OD measuring	Microplate reader (wavelength 450 nm)								
Calculations	Corresponding software (recommended)								

## SAFETY PRECAUTIONS

- this kit is for invitro diagnostic use only. The operator should be thoroughly follow the manual to obtain the reliable data. This instruction manual is valid only for the present kit with the listed composition. Any exchange of kit components is not allowed by CE regulations.
- do not use kits or components after expiry date stated on the label. take into consideration stability period for reconstituted reagents.
- do not mix or use together reagents of different lots.
- stop reagent is 1N HCL solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water and seek medical advice.
- source materials of human origin that were used in preparation of kit were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. How ever, no known laboratory test guarantees the absence of these viral agents. Therefore, all the kit components and patient's samples should be handled as potentially hazardous.
- As the kit contains potentially hazardous material, the following precautions should be observed:
  - \*Do not smoke, eat or drink while performing the assay.
  - \*Always use protective gloves.
  - \*Never pipette material by mouth.
  - \*In the case of spilling, wipe up the spills promptly and wash the affected area thoroughly with decontaminant.
- GLP and all general and individual regulations should be applied to the use of the kit.

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**2.2.** “GonadotropinEIA-FSH” reagents are sufficient for determination of 40 unknowns, 6 calibrators, 1 control and 1 TMB Substrate control in duplicates, providing that all the strips are used simultaneously.

*NOTE: If used partially, kit should be used within a month after opening.*

**2.3. Assay principle.** “GonadotropinEIA-FSH” is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of FSH molecule. One of these antibodies (anti- $\alpha$ -subunit) is conjugated with horseradish peroxidase; the other (anti- $\beta$ -subunit) is immobilized on inner surface of microwells. During the incubation FSH molecules from the serum sample bind to both immobilized antibodies and anti-FSH-peroxidase conjugate. Then the wells are washed with wash buffer to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate is in direct proportion to the FSH concentration in tested sample. During the incubation with TMB Substrate solution the coloring appears. The color intensity is in direct proportion to the FSH concentration in sample. Optical density of the solution in the wells is measured and the FSH concentration in the samples is calculated using calibration curve.

### 3. PERFORMANCE CHARACTERISTICS

**3.1. Specificity.** No cross-interaction of monoclonal antibodies against beta-FSH with LH, TSH and hCG was detected.

**3.2. Coefficient of variation (intra-assay precision)** between the results of FSH determination in the same sample is less than 8%.

**3.3. Linearity (Dilution test).** Dilution of serum sample containing predetermined FSH concentration with FSH-free serum leads to linear recovery of FSH in diluted samples in the concentration range between Calibrator №2 and Calibrator №6.

**3.4. Recovery.** To determine this parameter, equal volumes of control and Calibrator №3 were mixed. Then the correspondence between the calculated FSH concentration in the obtained sample and the measured concentration was determined. Recovery range is 90–110%.

**3.5. Detectability.** Minimal FSH concentration detectable by “GonadotropinEIA-FSH” assay is 0.3 mIU/ml.

**3.6. Expected values.** Serum samples collected between 9 and 11 a.m. from apparently healthy people, both males and females, at the age of 19–65, were assayed with “GonadotropinEIA-FSH” test kit (see Table1 for results). These limits should be considered as guidelines only.

Table 1.

Category	N	Mean (mIU/ml)	Range (mIU/ml)
<i>Females</i>			
Normally menstruating (19-35 years old)	120		
Follicular phase		4.6	1.8-11.3
Midcycle peak		7.9	4.9-20.4
Luteal phase		3.3	1.1-9.5
Postmenopausal (49-65 years old)	15	68.4	31.0-130
<i>Males (21-39 years old)</i>	40	3.9	1.0-11.8

**3.7.** It is highly recommended for each laboratory to determine its own reference range of FSH concentrations.

#### **4. WARNINGS AND PRECAUTIONS**

**4.1.** All the components are non-toxic.

**4.2.** Stop reagent is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water.

**4.3.** It is highly recommended to handle kit components in accordance with established good laboratory practice. The operator should wear disposable latex or plastic gloves and handle patients samples as if capable of transmitting infectious agents.

#### **5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:**

- digital variable pipettes that cover volume ranges from 0.005 to 0.05 ml; from 0.04 to 0.2 ml; from 0.2 to 1 ml and from 1 to 5 ml, with appropriate disposable tips;

- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;

- microplate shaker-thermostat, able to maintain temperature +37°C and shaking speed 500 to 800 rpm;;

- automatic microplate reader;

- volumetric cylinder;

- volumetric beaker;

- distilled water.

#### **6. REAGENT PREPARATION FOR ASSAY**

**6.1. FSH calibrators and control** are ready to use. Once opened, store at +2... 8°C for no more than 1 month.

**6.2. Microtitration strips.** Before opening keep the bag at room temperature (+18...25°C) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at +2...8°C until expiry date stated on the label.

**6.3 Wash buffer.** Prepare the necessary volume of Wash buffer by dilution of Buffer P 10-fold with distilled water. For example:

5 ml of Buffer P + 45 ml of distilled water. Mix thoroughly, avoiding foaming.

Keep firmly closed. Store at room temperature (+18...25°C) for no more than 5 days. The rest of the Buffer P should be stored firmly closed at +2...8°C until expiry date.

**6.4. Conjugate E** is ready to use. Once opened, store at +2...8°C for no more than 1 month.

**6.5. TMB Substrate solution** is ready to use. Once opened, store at +2...8°C for no more than 1 month.

**6.6. Stop reagent** is ready to use. Once opened, store at +2...8°C until expiry date.

## **7. ASSAY PROCEDURE**

**7.1.** All the kit components and serum samples should be brought to room temperature (+18...25°C) and stirred thoroughly before the assay. Assay scheme is given on the last page.

**7.2.** Mark the wells as follows:

A1, A2 — № 1 for TMB substrate control;

B1, B2 — № 2 for calibrator № 1;

C1, C2 — № 3 for calibrator № 2;

D1, D2 — № 4 for calibrator № 3;

E1, E2 — № 5 for calibrator № 4;

F1, F2 — № 6 for calibrator № 5;

G1, G2 — № 7 for calibrator № 6;

H1, H2 — № 8 for control.

**7.3.** Perform each assay in duplicate for both calibrators and unknowns.

**7.4.** Pipette 100 µl of Conjugate E into each well **except A1 and A2 wells**.

**7.5.** Pipette 50 µl of FSH calibrators, control and serum samples into the corresponding wells.

**7.6.** Incubate strips for 1 hour on shaker-thermostat at +37°C (500-800 rpm).

**7.7.** After incubation, aspirate the liquid from the wells and wash the strips five times with wash buffer. Each time add 300 µl of wash buffer per well and shake for 5–10 sec, then aspirate the buffer. After the last washing cycle, if necessary, invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

**7.8.** Add 100 µl of TMB Substrate solution into each well. Incubate at room temperature in the dark for 10 – 30 min depending on the color intensity.

**7.9.** Add 100 µl of Stop reagent to all wells and shake well for 1–2 minutes.

**7.10.** Read the optical density of the solution in the microwells at a wavelength of 450 nm.

**7.11.** Calculate the mean absorbency for each duplicate. Subtract the mean absorbency value of A1-A2 wells from the mean absorbency values of calibrators; control and unknown samples. Draw the calibration curve in linear coordinates by plotting absorbency values for calibrators against corresponding FSH concentrations. Determine FSH concentrations in the unknown samples and control.

**7.12.** An appropriate computer software may be used for calculations. Use the mean absorbency value of A1-A2 wells as “blank”.

## **8. PROCEDURAL NOTES**

**8.1.** “GonadotropinEIA-FSH” kit should be stored at + 2...8°C until expiry date stated on the label.

If used for separate experiments kit contents should be stored as follows:

- put remaining strips in plastic bag and close tightly. Keep at +2...8°C until expiry date;

- once opened, store Conjugate E and TMB substrate solution at + 2...8°C for no more than 1 month;
- once opened, store Buffer P at +2...8°C until expiry date;
- store prepared wash buffer firmly closed at room temperature for no more than 5 days;
- store calibrators and control at +2...+8°C for no more than 1 month after opening;
- store Stop reagent at +2...8°C until expiry date.

**8.2.** Do not use plasma, hemolysed or lipemic serum or samples with sodium azide as a preservative.

**8.3.** Please take into consideration that calibrators should be measured in each separate assay. It is also recommended to measure FSH concentration in the control each time. The number of separate experiments that can be performed with one kit (4 experiments) is therefore limited by the volume of calibrators.

**8.4.** Do not use Stop reagents from other manufacturers.

**8.5.** The operator should thoroughly follow the manual to obtain the reliable result.

### SCHEME OF ASSAY

Stage of assay and reagents used	Numeration of the wells (according to 7.2)								
	1	2	3	4	5	6	7	8	9-48
Conjugate E, µl	–	100	100	100	100	100	100	100	100
Calibrator № 1, µl	–	50	–	–	–	–	–	–	–
Calibrator № 2, µl	–	–	50	–	–	–	–	–	–
Calibrator № 3, µl	–	–	–	50	–	–	–	–	–
Calibrator № 4, µl	–	–	–	–	50	–	–	–	–
Calibrator № 5, µl	–	–	–	–	–	50	–	–	–
Calibrator № 6, µl	–	–	–	–	–	–	50	–	–
Control, µl	–	–	–	–	–	–	–	50	–
Unknown sample, µl	–	–	–	–	–	–	–	–	50
Incubation № 1	<b>1 hour on shaker-thermostat at +37°C</b>								
Wash (five times): wash buffer, µl	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300	5x 300
TMB solution, µl	100	100	100	100	100	100	100	100	100
Incubation № 2	<b>15-30 minutes in the dark at room temperature</b>								
Stop Reagent, µl	100	100	100	100	100	100	100	100	100
Stirring	<b>1-2 minutes on shaker</b>								
OD measuring	Microplate reader (wavelength 450 nm)								
Calculations	Corresponding software (recommended)								

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