Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

CA125 (Cancer Antigen) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIA1251	CA125 ELISA	96 Tests

Intended Use:

CA125 Elisa is intended to be used for the quantitative determination of CA125 in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

CA125 is a marker of ovarian cancer. 60% of women with CA125 values over 35 U/ml have been found to have ovarian carcinoma. Specificity of the test is increased by complimenting the CA125 assay along with a pelvic examination. Serum CA125 concentration is also used to check the prognosis of the disease and treatment efficacy. CA125 is the most sensitive marker of Ovarian cancer known as yet.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti-CA125 antibody. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry date at 2-8°C.
CA125 Calibrators	6x1.0ml	6 vials containing CA125 at concentrations of 0.0, 15, 50, 100, 200 and 400 U/ml made up in a human serum matrix. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable until expiry date at 2-8°C.
Enzyme Conjugate	1x12ml	1 vial containing 12 ml of HRP labelled monoclonal Anti-CA125 antibody in Buffered saline. Once open, stable until expiry date at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry date at 2-8°C.
Stop Solution	1x12ml	2N HCl. Ready to use. Once open, stable until expiry date at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. Avoid grossly haemolytic, lipaemic or turbid samples. Plasma samples collected in tubes containing EDTA, heparin or oxalate may interfere with the test procedures and should be avoided. Specimen 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.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag with desiccant provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until the labelled expiry date.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50 µl of Calibrators and Samples to each well. Mix thoroughly for 10 seconds.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of the Enzyme Conjugate to each well. Tap the plate for 30 seconds to mix. Complete mixing is important at this step.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 3 hours at 37°C.

STEP 5

Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 10 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

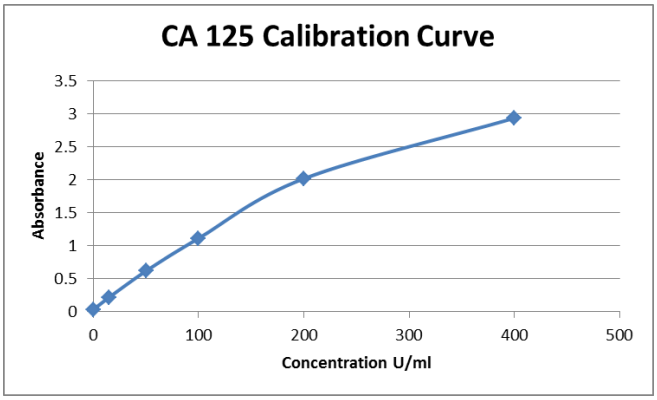
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. It is recommended that no more than 32 wells are used for each assay run if manual pipetting is used since pipetting of all calibrators, specimens, controls should be completed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

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in U/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.010	0.0 U/ml
CAL B	0.105	15.0 U/ml
CAL C	0.347	50.0 U/ml
CAL D	0.703	100.0 U/ml
CAL E	1.411	200.0 U/ml
CAL F	2.437	400.0 U/ml



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

Expected Values:

In healthy individuals CA125 values are generally below 35 U/ml.

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Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	20	16.27	0.970	5.96%
2	20	86.26	4.29	4.97%

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
1	10	17.19	1.698	9.87%
2	10	88.62	6.16	6.95%

3. **Sensitivity:**

The minimum detectable concentration of CA125 by this assay was found to be 5 U/ml.

Linearity:

Two patient sera were serially diluted with 0 U/ml standard in a linearity study. The average recovery was 99.0 %

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undiluted	218.02 U/ml	218.02 U/ml	
2x	109.01 U/ml	110.62 U/ml	101.5 %
4x	54.51 U/ml	53.02 U/ml	97.3 %
8x	27.25 U/ml	26.11 U/ml	95.8 %
16x	13.63 U/ml	14.80 U/ml	108.6 %
Average Recovery: 100.8 %			

Sample B			
Dilution	Expected	Observed	% Recovery
Sample undiluted	260.33 U/ml	260.33 U/ml	
2x	130.17 U/ml	128.23 U/ml	98.5 %
4x	65.08 U/ml	64.98 U/ml	99.8 %
8x	32.54 U/ml	31.76 U/ml	97.6 %
16x	16.27 U/ml	15.11 U/ml	92.9 %
Average Recovery: 97.2 %			

Method Comparison:

Method comparison between this assay and a commercially available assay yielded the following data:

N=62, Correlation Coeff: 0.981, Slope: 0.933, Intercept: 2.06

Mean values: This assay: 38.79 U/ml and comparator: 35.88 U/ml

References:

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2. Sakesela F. Prognostic markers in epithelial ovarian cancer. Intl J Gynecol Pathol, 1993; 12: 156-161.
3. Farghaly SA. Tumour markers in gynecologic cancer. Gynecol & Obstet Invest, 1992; 34: 65-72.
4. Olt G, Berchuck A and Bast RC. The role of tumour markers in gynecologic oncology
5. Diez M, Cerdan FJ, Ortega MB, Torres A, Picardo A, Balibrea JL. Evluation of Serum CA 125 as a tunor marker in non small cell lung cancer. Cancer, 1991; 67: 150-154.
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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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