FOR INFORMATION ONLY. WHEN PERFORMING THE ASSAY ALWAYS REFER TO PACKAGE INSERT SUPPLIED WITH THE KIT

CanAg CA125 EIA

REF 400-10

IVD

CE

Instructions for use. 2012-11

EN	EXPLANATION OF SYMBOLS
BG	ОБЯСНЕНИЕ НА СИМВОЛИТЕ
CS	VÝZNAM SYMBOLŮ
DA	SYMBOLFORKLARING
DE	ERKLÄRUNG DER SYMBOLE
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	SIGNIFICADO DE LOS SÍMBOLOS
ET	SÜMBOLITE SELGITUS
FR	EXPLICATION DES SYMBOLES
HR	OBJAŠNJENJE SIMBOLA
HU	JELMAGYARÁZAT
IT	SPIEGAZIONE DEI SIMBOLI
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	SIMBOLU SKAIDROJUMS
NL	VERKLARING DER SYMBOLEN
NO	SYMBOLFORKLARING
PL	OBJAŚNIENIE SYMBOLI
PT	EXPLICAÇÃO DOS SÍMBOLOS
RO	SEMNIFICAŢIA SIMBOLURILOR
RU	ОБОНАЧЕНИЯ
SV	SYMBOLFÖRKLARING
SK	VÝZNAM SYMBOLOV
SL	RAZLAGA SIMBOLOV
SR	OBJAŠNJENJE SIMBOLA
TR	SEMBOLLERİN AÇIKLAMALARI

Use By/Годно до/Použitelné do/Holdbar til/Verwendbar bis/Ημερομηνία λήξης/Fecha de caducidad/Kõlblik kuni/Utiliser jusque/Rok valjanosti/Felhasználható/Utilizzare entro/Sunaudoti iki/Izlietot līdz/Houdbaar tot/Brukes innen/Użyć przed/Prazo de validade/Expiră la/Использовать до/Använd före/Použite´né do/ Uporabno do/Upotrebljivo do/Son Kullanma Tarihi

LOT

Batch code/Номер на партида/Číslo šarže/Lotnummer/Chargenbezeichnung/Αριθμός Παρτίδας/Código de lote/Partii kood/Code du lot/Kod serije/Sarzsszám/Codice del lotto/Partijos kodas/Partijas kods/Lot nummer/Partikode/Kod partii/Código do lote/Număr de lot/Номер лота/Lotnummer/Číslo šarže/Številka serije/Kod partije/Parti Kodu

Date of manufacture/Дата на производство/Datum výroby/ Produktionsdato/Herstellungsdatum/ Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/ Date de fabrication/Datum proizvodnje/ Gyártási idő/Data di produzione/ Pagaminimo data/Ražošanas datums/ Productiedatum/Fremstillingsdato/ Data produkcji/Data de fabrico/Data fabricaţiei/Дата производства/ Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

Temperature limitation/ Температурни граници/ Teplotní omezení/ Temperaturbegrænsning/ Temperaturbegrenzung/ Περιορισμοί θερμοκρασίας/ Límites de temperatura/ Temperatuuri piirang/ Limite de température/ Temperaturno ograničenje/ Hőmérsékletre vonatkozó korlátozás/ Limiti di temperatura/ Temperatūriniai apribojimai/ Temperatūras ierobežojums/ Temperatuurbeperking/ Temperaturbegrensninger/ Temperatury graniczne/ Limite de temperatura/ Limite de temperatură/ Температурный режим/ Temperaturbegränsning/ Teplotné obmedzenie Omejitev temperature/ Temperaturno ograničenje/ Sıcaklık sınırlaması/

IVD

In Vitro Diagnostic Medical Device/ Медицински уред за диагностика ин витро/Diagnostický zdravotnický prostředek in vitro/Medicinsk udstyr til in vitro-diagnostik/In-vitro-Diagnostikum/ Ιατροτεχνολογικό προϊόν για διάγνωση In Vitro/Dispositivo médico para diagnóstico in vitro/In vitro diagnostiline meditsiiniseade/Dispositif médical de diagnostic in vitro/Diagnostički medicinski uređaj In Vitro/In vitro orvosdiagnosztikai eszköz/Dispositivo medico per test diagnostici in vitro/In Vitro Diagnostinė Medicinos Priemonė/Medicīniska ierīce in vitro diagnostikai/In vitro-diagnostisch medisch instrument/In vitro diagnostisk medisinsk utstyr/Wyrób medyczny do diagnostyki in vitro/Dispositivo Médico de Diagnóstico In Vitro/Dispozitiv medical pentru diagnostic in vitro/Только для диагностики In Vitro/Endast för in vitro-diagnostik/ Zdravotnícka pomôcka na diagnostiku in vitro/In vitro diagnostični pripomoček/Diagnostički medicinski uređaj In Vitro/<96> testleri için yeterlilik içerir

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilstrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιεχόμενο επαρκές για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour “96” tests/Sadrži dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per “96” saggi/Turinys skirtas atlikti <96> tyrimus/ Saturs pietiekams <96> testiem/Inhoud voldoende voor “96” testen/til “96” test/ Tilstrekkelig innhold for <96> prøver/ Wystarczy na wykonanie <96> testów/ Conteúdo suficiente para “96” ensaios/ Conţinut suficient pentru 96 de teste/ Содержит достаточные количества для «96» определений/Innehåller tillräckligt till “96” antal tester/Obsah postačuje na tento počet testov: <96>/Vsebina zadostuje za <96> testov/Sadržina dovoljna za <96> testova/<96> testleri için yeterlilik içerir

REF

Catalogue number/Каталожен номер/ Katalogové číslo/Katalognummer/ Bestellnummer/Αριθμός καταλόγου/ Número de catálogo/Kataloogi number/ Numéro de catalogue/Kataloški broj/ Katalógusszám/Numero di catalogo/ Katalogo numeris/Numurs katalogā/ Katalogusnummer/Katalognummer/ Numer katalogowy/Número do catálogo/ Număr de catalog/Номер по каталогу/ Produktnummer/Katalógové číslo/ Kataloška številka/Kataloški broj/ Katalog numarası

Consult Instructions for Use/ Прочетете инструкцията за употреба/Konzultujte s návodem k použití/Se brugsanvisning/Siehe Gebrauchsanweisung/Συμβουλευτείτε τις Οδηγίες σχετικά με τη χρήση/ Consulte las instrucciones de uso/ Vt kasutusjuhendit/Consulter le mode d'emploi/Pročitajte upute za uporabu/ Olvassa el a használati utasítást/ Consultare le istruzioni per l'uso/Dėl naudojimo žiūrėkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene før bruk/Sprawdzić w instrukcji użycia/ Consulte as Instruções de Utilização/ Consultaţi instrucţiunile de utilizare/ Обратитесь к инструкции по применению/Se bruksanvisning/ Prečítajte si návod na používanie/ Pročitajte uputstvo za upotrebu/ Kullanım Talimatlarına Bakınız

CONT

Contents of kit/Съдържание на набора/ Obsah soupravy/Kittets indhold/Inhalt des Kits/Περιεχόμενα του κιτ/Contenido del kit/Komplekt sisaldab/Contenu du kit/Sadržaj opreme/A készlet tartalma/ Contenuto del kit/Rinkinio turinys/ Komplekta saturs/Inhoud van de set/ Settets innhold/Zawartość zestawu/ Conteúdo do kit/Conţinutul setului/ Компоненты набора/Kit innehåll/ Obsah súpravy/Vsebina kompleta/Sadržaj opreme/Kitin içindekiler

Biological risks/Биологическа опасност/Biologická rizika/Biologisk fare/Biologische Gefahren/Βιολογικοί κίνδυνοι/Riesgos biológicos/ Bioloogilised ohud/Risques biologiques/ Biološki rizici/Biológiai kockázatok/Rischi biologici/Biologinis pavojus/Bioloģiskais risks/Biologische risico's/Biologiske risikoer/Zagrożenie biologiczne/Riscos biológicos/ Biologisk risk/Pericole biologice/Биологическая опасность/ Biologicky rizikové/Biologické riziká/ Biološki rizici/Biyolojik riskler

ORIG | HUM

Human/С човешки произход/Lidské/ Humant/Human/δείγματα αναφοράς/ Humano/Inimpäritolu/Humaine/Ljudskog porjekla/Humán/Origine Umana/ Žmogaus kilmės/Cilvēku izcelsmes/ Human/Menneske/Ludzka/Humano/ Origine umană/Человеческого происхождения/Human/Ľudské/ Humanega izvora/Ljudskog porekla/İnsan

ORIG | MOU

From mouse/С миши произход/Myší/ Fra mus/Maus/απο ποντίκι/de ratón/ Hiirtelt/De souris/Mišiej porjekla/ Egérből/Murino/Pelės kilmės/No peles/ Van muizen/Fra mus/Mysia/Do rato/De la şoareci/Мышиного происхождения/ Från mus/Myšie/Mišjega izvora/Mišiej porekla/Fareden

ORIG | BOV

Bovine/С говежди произход/Hovězí/ Bovin/Rind/από βοοειδή/Bovino/ Veistelt/Bovine/Rogate stoke/Szarvasmarha/Bovino/Jaučio/No liellopa/ Bovien/Bovin/Wołowy/Bovino/Origine bovină/крупного рогатого скота/Från ko/Hovädzie/Govejega izvora/Rogate krupne stoke/Bovin

Reconstitute with/Разтваряне с/ Rozřeďte pomocí/Rekonstitueres med/ Rekonstituieren mit/Ανασύσταση με/ Reconstituir con/Lahjendamine/ Reconstituer avec/Rekonstituirajte s/ Feloldáshoz/Ricostituire con/Atkurti, ištirpdant su/Atšķaidīt ar/Reconstitutie met/Rekonstitueres med/Odtworzyć za pomocą/Reconstituir com/A se reconstitui cu/Растворить в/ Rekonstituera med/Rozrieďte pomocou/ Rekonstituirajte z/s/Ponovno formiranje sa/Yeniden oluşturulur

Manufacturer/Производител/Výrobce/ Producent/Hersteller/Κτασκευαστής/ Fabricante/Tootja/Fabricant/Proizvođač/ Gyártó/Fabbricante/Gamintojas/ Ražotājs/Fabrikant/Produsent/ Producent/Fabricante/Producător/ Производитель/Tillverkare/ Výrobca/ Izdelovalec/Proizvođač/Üretici

EN

CanAg CA125 EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CA125 EIA kit is intended for the quantitative determination of the cancer associated antigen CA125 in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

CA125 is a high molecular weight mucin type glycoprotein, originally defined by the Oc125 monoclonal antibody (MAb) established by Bast et al. (1). Different epitopes, co-expressed with the Oc125 epitope on the CA125 antigen, have been used for the development of heterologous assays for determination of the CA125 antigen (2). The CanAg CA125 EIA is based on two mouse monoclonal antibodies, Ov197 and Ov185, directed against two independent epitopes of the protein core of the CA125 antigen (3, 4).

Assays for CA125 are frequently used to monitor patients with gynecological malignancies such as epithelial ovarian cancer (5).

PRINCIPLE OF THE TEST

The CanAg CA125 EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, controls and patient samples are incubated together with a biotinylated Anti-CA125 monoclonal antibody (MAb) Ov197 (derived from mice) in streptavidin coated microstrips. CA125 present in calibrators or samples is adsorbed to the streptavidin coated microstrips by the biotinylated Anti-CA125 MAb during the incubation. The strips are then washed and incubated with HRP labeled Anti-CA125 MAb Ov185. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methyl-benzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color is proportional to the amount of CA125 present in the samples.

The color intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution).

Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CA125 concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA125 EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8 °C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2–8 °C immediately after use.

Component	Quantity	Storage and stability after first opening
MICROPLA		
Microplate	1 Plate	2–8 °C until expiry date stated on the plate
12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.		
CA125 Calibrators	5 vials	2–8 °C until expiry date stated on the vials
CAL CA125 0	0 U/mL	1 x 8 mL
CAL CA125 10	10 U/mL	1 x 0.75 mL
CAL CA125 40	40 U/mL	1 x 0.75 mL
CAL CA125 200	200 U/mL	1 x 0.75 mL
CAL CA125 500	500 U/mL	1 x 0.75 mL
CA125 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, detergent, an inert yellow dye, and 0.05 % sodium azide as preservative. Ready for use. CAL CA125 0 should also be used for dilution of samples.		

Component	Quantity	Storage and stability after first opening
CA125 Controls	2 vials	2–8 °C until expiry date stated on the vials
CONTROL | CA125 | 1	1 x 0.75 mL	
CONTROL | CA125 | 2	1 x 0.75 mL	

CA125 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, detergent, and 0.05 % sodium azide as preservative. Ready for use.

BIOTIN | Anti-CA125		
Biotin Anti-CA125	1 x 15 mL	2–8 °C until expiry date stated on the vial

Biotin Anti-CA125 monoclonal antibody from mouse, approximately 2 µg/mL. Contains Tris-HCl buffered saline (pH 7.75), bovine serum albumin, blocking agents, detergents, an inert red dye, and 0.05 % sodium azide as preservative. Ready for use.

CONJ | Anti-CA125		
Tracer, HRP Anti-CA125	1 x 0.75 mL	2–8 °C until expiry date stated on the vial

Stock Solution of HRP Anti-CA125 monoclonal antibody from mouse, approximately 30 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL | CONJ		
Tracer Diluent	1 x 15 mL	2–8 °C until expiry date stated on the vial

Phosphate buffered saline (pH 7.2) with bovine serum albumin, blocking agents, detergents, an inert blue dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
SUBS | TMB		
TMB HRP-Substrate	1 x 12 mL	2–8 °C until expiry date stated on the vial
Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine (TMB). Ready for use.		
STOP		
Stop Solution	1 x 15 mL	2–8 °C until expiry date stated on the vial
Contains 0.12 M hydrochloric acid. Ready for use.		
WASHBUF | 25X		
Wash Concentrate	1 x 50 mL	2–8 °C until expiry date stated on the bottle
A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with water 25 times before use.		

Indications of instability

The TMB HRP-Substrate should be colorless or slightly bluish. A blue color indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use

- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88–8395 on laboratory safety procedures or any other local or national regulation.
- Handle all patient specimens as potentially infectious.
- Reagents contain sodium azide (NaN_3) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
- Follow local guidelines for disposal of all waste material.

Caution

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV 1 and 2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

SPECIMEN COLLECTION AND HANDLING

The CanAg CA125 EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8 °C for 24 hours. For longer periods store samples at -70 °C or below. Samples should not be stored in a self-defrosting freezer and not be thawed and refrozen before analysis. Allow frozen samples to thaw slowly at 2–8 °C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous, approximately 900-1100 oscillations/min.
2. **Microplate wash device**
   Automatic plate wash capable of performing 1, 3 and 6 washing cycles, with a minimal fill volume of 350 µL/well/washcycle.
   The Nunc Immuno-8 manual strip washer is recommended if an automatic microplate washer is not used.
3. **Microplate spectrophotometer**
   With a wavelength of 620 nm and/or 405 nm, and an absorbance range of 0 to 3.0.
4. **Precision pipettes**
   With disposable plastic tips for dispensing microlitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential. Pipettes for dispensing millilitre volumes.
5. **Distilled or deionized water**
   For preparation of Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg CA125 EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25 °C) prior to use. The assay should only be performed at temperatures between 20–25 °C to obtain accurate results. Frozen specimens must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and unknown specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.
   - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. It's highly recommended to use *plate* process mode and *overflow* wash mode with a dispensing volume of 800 µL. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
5. The TMB HRP-Substrate is very sensitive to contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial into a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or dispenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper precision pipetting technique when handling samples and reagents. Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over. A diligent pipetting technique is of particular importance when handling the samples and the TMB HRP-Substrate solution.

Protocol Sheet

CanAg CA125 EIA REF 400-10

Mix the components directly before use. Use shaking conditions according to the Instructions.

Step		Vial/Plate	Procedure
1.	Prepare Wash Solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionized water.
	Prepare Tracer working solution	CONJ Anti-CA125 DIL CONJ	Mix 50 µL of Tracer, HRP Anti-CA125 with 1mL of Tracer Diluent per strip:
2.	Wash	MICROPLA	Wash each well once with Wash Solution. Use manual or automatic washer.

No. of Strips	Tracer, HRP Anti-CA125 (µL)	Tracer Diluent (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

3.	Add calibrators, controls and samples	CAL | CA125 0, 10, 40, 200, 500 CONTROL | CA125 1, 2	25 µL in each well
4.	Add Biotin Anti-CA125	BIOTIN | Anti-CA125	100 µL in each well
5.	Incubate	MICROPLA	2 hour shaking at room temperature
6.	Wash	MICROPLA	Wash each well three times with Wash Solution. Use manual or automatic washer.
7.	Add Tracer working solution	TRACER WORKING SOLUTION	100 µL in each well
8.	Incubate	MICROPLA	1 hour shaking at room temperature
9.	Wash	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.
10.	Add TMB HRP-Substrate	SUBS | TMB	100 µL in each well
11.	Incubate	MICROPLA	30 min shaking at room temperature
12.	Read absorbance	MICROPLA	620 nm
Alt.12	Add Stop Solution	STOP	100 µL in each well
Alt.13	Incubate	MICROPLA	1 min shaking at room temperature
Alt.14	Read absorbance	MICROPLA	Read at 405 nm within 15 min

Preparation of reagents	Stability of prepared reagent
Wash Solution	2 weeks at 2–25 °C in a sealed container

Pour the 50 mL Wash Concentrate into a clean container and dilute 25-fold by adding 1200 mL of distilled or deionised water to give a buffered Wash Solution.

Tracer working solution	3 weeks at 2–8 °C in a sealed container

Prepare the required quantity of Tracer working solution by mixing 50 µL of Tracer, HRP Anti-CA125 with 1 mL of Tracer Diluent per strip (see table below):

No. of Strips	Tracer, HRP Anti-CA125 (µL)	Tracer Diluent (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of Tracer working solution.

Alternative: Pour the content of the Tracer, HRP Anti-CA125 into the vial of Tracer Diluent and mix gently. Make sure that the entire content of the Tracer, HRP Anti-CA125 is transferred to the vial of Tracer Diluent.

NOTE: The Tracer working solution is stable for 3 weeks at 2–8 °C. Do not prepare more Tracer working solution than will be used within this period and make sure that it is stored properly.

Assay procedure

Perform each determination in duplicate for both calibrators and patient samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25 °C) before use.

1. Start to prepare Wash Solution and Tracer working solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 25 µL of the CA125 Calibrators (CAL 0, 10, 40, 200, 500), CA125 Controls (C 1, C 2) and patient specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 500	2nd Unk				
B	Cal 0	Cal 500	2nd Unk				
C	Cal 10	C1	etc.				
D	Cal 10	C1					
E	Cal 40	C2					
F	Cal 40	C2					
G	Cal 200	1st Unk					
H	Cal 200	1st Unk					

4. Add 100 µL of Biotin Anti-CA125 to each well using a 100 µL precision pipette (or an 8-channel 100 µL precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid.
5. Incubate the plate for 2 hours (± 10 min) at room temperature (20-25 °C) with constant shaking of the plate using a microplate shaker.

6. After the first incubation aspirate and wash each strip 3 times using the wash procedure described in Procedural notes, item 4.
7. Add 100 µL of Tracer working solution to each well. Use the same pipetting procedure as in item 4 above.
8. Incubate the frame for 1 hour (± 5 min) at room temperature (20–25 °C) with constant shaking.
9. After the second incubation aspirate and wash each strip 6 times, using the wash procedure described in Procedural notes, item 4.
10. Add 100 µL of TMB HRP-Substrate to each well using the same pipetting technique as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between addition to the first and last well should not exceed 5 min.
11. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid exposure to direct sunlight.
12. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

Option

If the laboratory does not have access to a microplate reader capable of reading at 620 nm, the absorbance can be determined as in item alternative 12:

Alt. 12. Add 100 µL of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg CA125 EIA measures concentrations between 1.5 and 500 U/mL. If CA125 concentrations above the measuring range are to be expected, it is recommended to dilute samples with CA125 Calibrator 0 prior to analysis.

Quality control

CA125 Control 1 and 2 should be used for validation of the assay series. Ranges of expected results are indicated on the vial labels. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated. Each laboratory may also prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

Reference materials

Since no common reference material is available for CA125 antigen, CanAg CA125 EIA Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer with built-in data calculation program is used, refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the CA125 Calibrators.
For automatic calculation of CA125 results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 U/mL.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 U/mL.

NOTE: 4-Parametric or Linear regression evaluation methods should not be used.

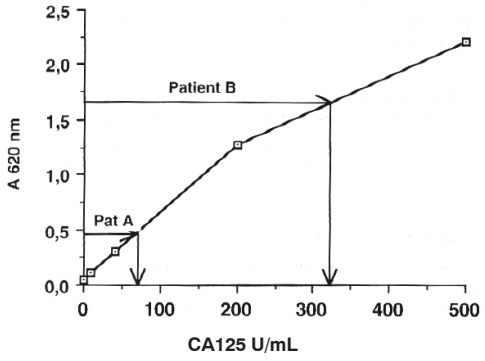
For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CA125 Calibrator against the corresponding CA125 concentration (in U/mL), see figure below. The unknown CA125 concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

If samples in an initial analysis give CA125 levels higher than 500 U/mL the samples should be diluted 1/10 and 1/100 with CA125 Calibrator 0 to obtain the accurate CA125 concentration of the samples.
1/10 dilution = 50 µL of specimen + 450 µL of CA125 Calibrator 0
1/100 dilution = 50 µL of 1/10 dilution + 450 µL of CA125 Calibrator 0

The CA125 concentration of the undiluted sample is then calculated as:
Dilution 1/10: 10 x measured value
Dilution 1/100: 100 x measured value

Example of results

Specimen			Calibrator values	Mean abs value (A)	CA125 U/mL
CAL	CA125	0	0 U/mL	0.047	
CAL	CA125	10	10 U/mL	0.116	
CAL	CA125	40	40 U/mL	0.298	
CAL	CA125	200	200 U/mL	1.269	
CAL	CA125	500	500 U/mL	2.218	
Specimen A				0.490	69.8
Specimen B				1.650	325



Example, do not use this curve to determine assay results.

LIMITATIONS OF THE PROCEDURE

The level of CA125 cannot be used as absolute evidence for the presence or absence of malignant disease and the CA125 test should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the management of patients, and the CA125 test should not replace any established clinical examination.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffers.

EXPECTED VALUES

CanAg CA125 was measured in 100 healthy female blood donors. The mean value obtained was 14.7 U/mL with a standard deviation of 7.7. The median value was 13.1 U/mL, range 5.06– 47.9 U/mL. The lower and upper extremes of the normal range were examined using IFCC recommended non-parametric statistical treatment. The reference interval contains the central 95% fraction of the reference distribution. Reference limits may accordingly be estimated as the 2.5% (lower) and 97.5% (upper) fractiles. These limits cut off a fraction of 2.5% of the values in each tail of the reference distribution. Non-parametric estimates:
N=100

Fraction	Reference limit (U/mL)
2.5th (lower)	5
97.5th (upper)	39

96% of the healthy women had assay values below 35 U/mL.
It is recommended that each laboratory establish its own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc. It should also be borne in mind that the individual patient's own baseline result provides the most important reference point for interpretation of marker results (6).

PERFORMANCE CHARACTERISTICS

Precision

Total precision was determined according to NCCLS guideline EP5-A (7) using four levels of frozen pooled human serum containing added ascitespool. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 10 days. The analyses were undertaken during a period of 8 months, by ≥ two different technicians and using 10 different CanAg CA125 EIA kit batches.

Sample	Replicates	Mean U/mL	Within-run SD (U/mL)	Within-run CV %	Between-day SD (U/mL)	Between-day CV %
CA125 1	40	16.8	0.74	4.4	0.53	3.1
CA125 2	40	75.7	3.26	4.3	2.42	3.2
CA125 3	40	201	8.55	4.3	7.58	3.8
CA125 4	40	392	11.4	2.9	15.5	4.0

Detection limit

The detection limit of the CanAg CA125 EIA assay is < 1.5 U/mL defined as the concentration corresponding to the mean of the absorbance values for the CA125 Calibrator 0 plus 2 standard deviations according to the formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 10 - OD\ CAL\ 0} \times 10\ U/mL$$

Recovery

Spiked serum samples were prepared by adding human CA125 antigen to normal serum samples. The recovery of the antigen was 100 ±15 %.

Hook effect

No hook effect has been noticed with samples up to 50 000 U/mL. **NOTE:** In very high samples the color of the substrate will change from blue to greenish (and eventually yellow in extremely high samples). This will lead to a falsely low absorbance at 620 nm, and in extreme cases the absorbance may fall within the calibration curve range and noticed as a hook.

Linearity

Patient samples were serially diluted with CA125 Calibrator 0 and analyzed. The obtained values were 100 ±15 % of the expected values.

Specificity

The CanAg CA125 EIA is based on two mouse monoclonal antibodies, Ov197 and Ov185, directed against two independent epitopes of the protein core of the CA125 antigen (4). The NCCLS guideline EP7-P (8) was followed to determine possible sources of interference. The following substances and concentrations were tested and found not to interfere with the test.

	Concentration with no significant (± 10 %) interference
Lipemia (Intralipid®)	4 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	5 mg/mL

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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