



CanAg CA242 EIA

**For Research Use Only.
Not for use in
diagnostic procedures.**

REF 101-85

Instructions for use. 2006-10

Enzyme immunometric assay kit
For 96 determinations

GB EXPLANATION OF SYMBOLS
DE BEDEUTUNG DER SYMBOLE
ES EXPLICACIÓN DE SÍMBOLOS
IT SIGNIFICATO DEI SIMBOLI
FR EXPLICATION DES SYMBOLES
DK SYMBOLFORKLARING
GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
SE SYMBOLFÖRKLARING



Use By/Verwendbar bis/
Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Holdbar til/Hμερομηνία λήξης/
Bäst före datum

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Batch code/Chargenbezeichnung/
Codigo de lote/
Codice del lotto/Code du lot/
Lotnummer/Αριθμός Παρτίδας/
Lotnummer



Date of manufacture/
Herstellungsdatum/
Fecha de fabricación/
Data di fabbricazione/
Date de fabrication/
Produktionsdato/
Ημερομηνία Παραγωγής/
Tilverkningsdatum

REF

Catalogue number/Bestellnummer/
Número de catálogo/
Numero di catalogo/
Référence du catalogue/
Katalognummer/
Αριθμός καταλόγου/
Produktnummer



Manufacturer/Hersteller/Fab-
ricante/Fabbricante/Fabricant/
Πroducent/τασκευαστής/
Tilverkare



Contains sufficient for <96> tests/
Ausreichend für "96" Ansätze/
Contenido suficiente
para <96> ensayos/
Contenuto sufficiente per "96" saggi/
Contenu suffisant pour "96" tests/
Indeholder tilstrækkeligt
til "96" test/
Περιεχόμενο επαρκές
για «96» εξετάσεις/
Innehåller tillräckligt till "96" tester



Temperature limitation/
Zulässiger Temperaturbereich/
Limite de temperatura/
Limiti di temperatura/
Limites de température/
Temperaturbegrænsning/
Περιορισμοί θερμοκρασίας/
Temperaturgräns



Consult Instructions for Use/
Gebrauchsanweisung beachten/
Consulte las instrucciones de uso/
Consultare le istruzioni per l'uso/
Consulter les instructions d'utilisation/
Se brugsanvisning/
Συμβουλευτείτε τις οδηγίες
χρήσης/
Se bruksanvisning



Biological risks/Biogefährdung/
Riesgo biológico/Rischio biologico/
Risques biologiques/
Biologisk fare/Βιολογικοί κίνδυνοι/
Biologisk risk



Contents of kit/Inhalt/Contenido/
Contenido/Contenu/Inndhold/
ανιδραστήρια/Kit innehåll



From mouse/der Maus/de ratón/
Murino/De souris/Mus/απο ποντίκι/
Från mus



Human/Human/Humano/
Origine Humana/Humaine/Human
δείγματα αναφοράς/Human

INTENDED USE

The CanAg CA242 EIA kit is intended for the quantitative determination of CA242 cancer antigen in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The tumor marker CA242 is defined by the monoclonal antibody C242. The chemical structure of the antigenic determinant is not exactly known, but the determinant have been shown to be a sialylated carbohydrate structure. In serum, CA242 is found on the same mucin-complex as CA50 and sialylated Lewis^a (CA19-9). Thus, CA242 is related, but not identical to the epitope of CA19-9 (1, 2).

PRINCIPLE OF THE TEST

The CanAg CA242 EIA is a solid-phase, non-competitive immunoassay based on the direct sandwich technique. Calibrators, controls and samples are incubated together with biotinylated anti-CA242 monoclonal antibody (MAb) C241 in Streptavidin coated microstrips. CA242 present in calibrators, controls or samples is adsorbed to the Streptavidin coated microstrips by the biotinylated anti-CA242 MAb during the incubation (3). The strips are then washed and incubated with horseradish peroxidase (HRP) labelled Anti-CA242 MAb C242. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportional to the amount of CA242 antigen present in the samples.

The colour 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 CA242 concentrations of samples are then read from the calibration curve.

REAGENTS

- Each CanAg CA242 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 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

Component	Quantity	Storage and stability after first opening
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CA242 Calibrators 5 vials 2–8°C until expiry date stated on the vials

CAL	CA242	0	0 U/mL	1 x 0.75 mL
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CAL	CA242	15	15 U/mL	1 x 0.75 mL
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CAL	CA242	50	50 U/mL	1 x 0.75 mL
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CAL	CA242	100	100 U/mL	1 x 0.75 mL
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CAL	CA242	150	150 U/mL	1 x 0.75 mL
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Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye, and 0.05% NaN₃ as preservative. Ready for use.

CA242 Controls 2 vials 2–8°C until expiry date stated on the vials

CONTROL	CA242	1	1 x 0.75 mL
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CONTROL	CA242	2	1 x 0.75 mL
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Human CA242 antigen in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.05% NaN₃ as preservative. Ready for use.

BIOTIN	Anti-CA242
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Biotin Anti-CA242 1 x 15 mL 2–8°C until expiry date stated on the vial

Biotin Anti-CA242 monoclonal antibody from mouse, approximately 1.5 µg/mL. Contains Tris-HCl buffered saline (pH 7.75) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert red dye and 0.05% NaN₃ as preservative. Ready for use.

Component	Quantity	Storage and stability after first opening
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CONJ	Anti-CA242
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Tracer, HRP Anti-CA242	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock solution of HRP Anti-CA242 monoclonal antibody from mouse, approximately 40 µg/mL. Contains preservatives. To be diluted with Tracer Diluent prior to use.

DIL	CONJ
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Tracer Diluent	1 X 15 mL	2–8° C until expiry date stated on the vial
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Phosphate buffered saline (pH 7.2) with bovine serum albumin, bovine immunoglobulin, blocking agents, detergent, an inert blue dye, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

SUBS	TMB
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TMB HRP-Substrate	1 x 12 mL	2–8°C until expiry date stated on the vial
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Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

STOP

STOP Solution	1 x 15 mL	2–8°C until expiry date stated on the vial
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Contains 0.12 M hydrochloric acid. Ready for use.

WASHBUF	25X
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Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
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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 colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

- Please refer to the US Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all 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-1/2 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 CA242 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 it is recommended to store the samples at –20° C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably 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. Longitudinal shaking approximately 200 strokes/min, oscillations 700-900/min.

2. **Microplate wash device**

Automatic platewash capable of performing 1, 3 and 6 washing cycles, or a semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid.

The Nunc Immuno-8 manual strip washer is recommended if an automatic microplatewash 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 to deliver microlitre and millilitre volumes.

An 8-channel pipette or resenser pipette with disposable plastic tips for delivery of 100 μ L is useful but not essential.

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 CA242 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 (20–28°C) prior to use. The assay should only be performed at temperatures between 20–28°C to obtain accurate results. Frozen specimens should be brought to room temperature slowly and must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators, controls and specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. A careful washing procedure of the strips is essential. Ensure that each well is filled up completely to the top edge and that the aspiration of the wells between and after the washing cycles is complete and the wells are dry. If there is liquid left in the wells, invert the plate and tap it carefully against absorbing paper.

Automatic strip washer: Follow the manufacturer's instructions for maintenance and wash the required number of wash cycles prior to and after each incubation step.

The aspiration/wash device should not be left standing with the Wash Solution for long periods as the needles may get clogged, giving poor liquid delivery and suction.

Protocol Sheet

CanAg CA242 EIA REF **101-85**

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

Step	Bottle/Plate	Procedure																																						
1. Prepare Wash Solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionized water.																																						
	CONJ Anti-CA242																																							
Prepare Tracer working solution	DIL CONJ	Mix 50 μ L of Tracer, HRP Anti-CA242 with 1 mL of Tracer Diluent per strip:																																						
			<table border="1"><thead><tr><th>No. of Strips</th><th>Tracer, HRP Anti-CA242 (μL)</th><th>Tracer Diluent (mL)</th></tr></thead><tbody><tr><td>1</td><td>50</td><td>1</td></tr><tr><td>2</td><td>100</td><td>2</td></tr><tr><td>3</td><td>150</td><td>3</td></tr><tr><td>4</td><td>200</td><td>4</td></tr><tr><td>5</td><td>250</td><td>5</td></tr><tr><td>6</td><td>300</td><td>6</td></tr><tr><td>7</td><td>350</td><td>7</td></tr><tr><td>8</td><td>400</td><td>8</td></tr><tr><td>9</td><td>450</td><td>9</td></tr><tr><td>10</td><td>500</td><td>10</td></tr><tr><td>11</td><td>550</td><td>11</td></tr><tr><td>12</td><td>600</td><td>12</td></tr></tbody></table>	No. of Strips	Tracer, HRP Anti-CA242 (μ L)	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
No. of Strips	Tracer, HRP Anti-CA242 (μ L)	Tracer Diluent (mL)																																						
1	50	1																																						
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6	300	6																																						
7	350	7																																						
8	400	8																																						
9	450	9																																						
10	500	10																																						
11	550	11																																						
12	600	12																																						
2. Wash	MICROPLA	Wash each well once with Wash Solution. Use manual or automatic washer.																																						

3. Add calibrators, controls and samples	<table border="1"> <tr> <td data-bbox="10 713 36 859">CAL</td> <td data-bbox="36 713 62 859">CA242</td> </tr> <tr> <td data-bbox="10 640 36 713">0, 15, 50, 100, 150</td> <td data-bbox="36 640 62 713">CONTROL</td> </tr> <tr> <td data-bbox="10 859 36 873">1, 2</td> <td data-bbox="36 859 62 873">CA242</td> </tr> </table>	CAL	CA242	0, 15, 50, 100, 150	CONTROL	1, 2	CA242	25 μ L in each well
CAL	CA242							
0, 15, 50, 100, 150	CONTROL							
1, 2	CA242							
4. Add Biotin Anti-CA242	BIOTIN Anti-CA242	100 μ L in each well						
5. Incubate	MICROPLA	2 hours 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 μ L 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 μ L 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 μ L 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						

- The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to 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 respenser pipette tip).
- Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. 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. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate.

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 deionized 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-CA242 with 1 mL of Tracer Diluent per strip (see table below):	

No. of Strips	Tracer, HRP Anti-CA242 (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 the Tracer Working Solution.

Alternative: Pour the content of the Tracer, HRP Anti-CA242 into the vial of Tracer Diluent and mix gently. Make sure that all of the Tracer, HRP Anti-CA242 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 calibrators, controls and samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–28°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 CA242 Calibrators (CAL 0, 15, 50, 100, 150), Controls (C1, C2) and samples (unknowns Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 150	Unk 2				
B	Cal 0	Cal 150	Unk 2				
C	Cal 15	C1	etc.				
D	Cal 15	C1					
E	Cal 50	C2					
F	Cal 50	C2					
G	Cal 100	Unk 1					
H	Cal 100	Unk 1					

4. Add 100 μL of Biotin Anti-CA242 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 the surface of the liquid.
5. Incubate the frame containing the strips for 2 hours (± 10 min) at room temperature (20–28°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 (20–28°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 procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the 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 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 spectrophotometer capable of reading at 620 nm, the absorbance can be determined as follows:

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

Measurement range

The CanAg CA242 EIA measures concentrations between 1 and 150 U/mL. If CA242 concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human serum prior to analysis. **NOTE:** The serum used for dilution must also be measured in order to determine the endogenous CA242 concentration (see “Calculation of results”).

Quality control

CA242 Control 1 and 2 may 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 in addition 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 material

Since no common reference material is available for CA242 antigen, CanAg CA242 EIA Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer reader with built-in data calculation program is used, refer to the manual for the plate reader and create a program using the concentration stated on the labels of each of the CA242 Calibrators.

For automatic calculation of CA242 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 should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CA242 Calibrator against the corresponding CA242 concentration (in U/mL), see figure below. The unknown CA242 concentrations can then be read from the calibration curve using the mean absorbance value of each specimen.

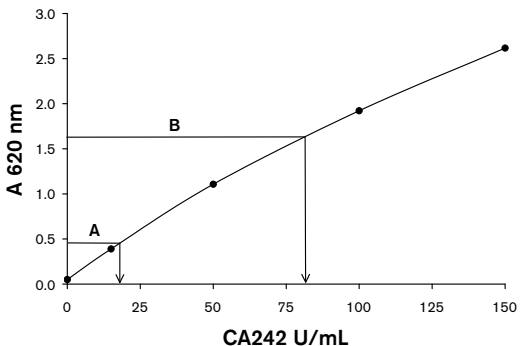
If samples in an initial analysis give CA242 levels higher than 150 U/mL, the samples should be diluted 1/10 with normal human serum and reanalysed to obtain the accurate CA242 concentration. **NOTE:** The sample used for dilution must also be measured in order to determine the endogenous CA242 concentration.

The CA242 concentration of the undiluted sample is calculated as:

$$\text{Dilution 1/10: } 10 \times ([\text{CA242}]_{\text{Diluted sample}} - (0.9 \times [\text{CA242}]_{\text{Normal serum}}))$$

Example of results

Specimen			Calibrator values	Mean abs value (A)	CA242 (U/mL)
CAL	CA242	0	0 U/mL	0.050	
CAL	CA242	15	15 U/mL	0.390	
CAL	CA242	50	50 U/mL	1.107	
CAL	CA242	100	100 U/mL	1.922	
CAL	CA242	150	150 U/mL	2.617	
Specimen A				0.410	16.1
Specimen B				1.636	80.9



Example (do not use this curve or table above to determine actual assay results).

LIMITATIONS OF THE PROCEDURE

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

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 event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

LITERATURE REFERENCES

1. Johansson, C., Nilsson, O., Bäckström, D., Jansson, E.-L. and Lindholm, L. (1991) Novel Epitopes on the CA50-Carrying Antigen: Chemical and Immunochemical Studies, *Tumor Biol.*, 12, 159-179.
2. Johansson, C., Nilsson, O. and Lindholm, L., (1991) Comparison of Serological Expression of Different Epitopes on the CA50-Carrying Antigen CanAg, *Int. J. Cancer*, 48, 757-763.
3. Dahlén U., Karlsson B., Lindholm L., Nilsson O., (1993) Development of an enzyme immuno-assay for determination of the tumour associated antigen CA242, *J. Tumor Marker Oncology* 8, 3, p 111.



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