

Product information

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CA72-4 ELISA

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For research use only, not for use in diagnostic procedures.

REF

IB79182



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CONTENTS

1 INTRODUCTION3
2 PRINCIPLE OF THE TEST*3
3 WARNINGS AND PRECAUTIONS4
4 REAGENTS5
5 SAMPLE COLLECTION AND PREPARATION7
6 ASSAY PROCEDURE7
7 QUALITY CONTROL8
8 PERFORMANCE CHARACTERISTICS9
9 LIMITATIONS OF USE10
10 LEGAL ASPECTS10
SYMBOLS USED WITH IBL-AMERICA ASSAYS12

1 INTRODUCTION

1.1 Intended Use

The **IBL-America TM-CA 72-4 ELISA** is an enzyme immunoassay for the measurement of CA 72-4 (TAG-72) in serum and plasma. **For research use only – Not for use in diagnostic procedures.**

2 PRINCIPLE OF THE TEST*

The IBL-America TM-CA 72-4 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal mouse antibody (Clone CC49) directed towards a unique antigenic site on a CA 72-4 molecule. An aliquot of sample containing endogenous CA 72-4 is incubated in the coated well with enzyme conjugate, which is an anti-CA 72-4 antibody (Clone B72.3) conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of CA 72-4 in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CA 72-4 in the sample.

* The antibodies used in this assay are patented by:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled "Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer" (**HHS** Reference No. E-185-1981/0-US-01)

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable latex gloves when handling samples and reagents. Microbial contamination of reagents or samples may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 1 N acidic solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from IBL-America.

4 REAGENTS

4.1 Reagents provided

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-CA 72-4 antibody (monoclonal).
2. **CAL 0 - 4 Standard (Standard 0-4)**, 5 vials, 0.5 mL, ready to use. Concentration: 0, 3, 20, 50, 100 U/mL
Contain non-mercury preservative.
3. **CONTROL low & high LYO Control Low & High**, 2 vials, (lyophilized) 0.5 mL each, see „Reagent Preparation“. Control values and ranges please refer to vial label or QC-Datasheet. Contains non-mercury preservative.
4. **SAM DIL Sample Diluent**, 1 vial, 3 mL, ready to use, Contains non-mercury preservative.
5. **ENZ CONJ 10x Enzyme Conjugate 10X concentrate**, 1 vial, 1.4 mL, anti-CA 72-4 antibody conjugated to horseradish peroxidase; see „Reagent Preparation“ Contains non-mercury preservative.
6. **ENZ CONJ DIL Conjugate Diluent**, 1 vial, 14 mL, ready to use. Contains non-mercury preservative.
7. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
8. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 1 N acidic solution, Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **WASH SOLN 40x Wash Solution**, 1 vial, 30 mL (40X concentrated), see „Preparation of Reagents“.

Note: Additional Sample Diluent for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2-8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8 °C. Microtiter wells must be stored at 2-8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Control

Reconstitute the lyophilized content with 0.5 mL distilled water and let stand for 10 minutes in minimum. Mix the controls several times before use. **Note:** The reconstituted controls should be appor- tioned and stored at -20 °C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash So- lution with 1170 mL distilled water to a final volume of 1200 mL. *The diluted Wash Solution is stable for 2 weeks at room temperature (20 °C to 26 °C).*

Enzyme Conjugate

Dilute *Enzyme Conjugate* concentrate 1:10 in *Conjugate Diluent*. *Stability of the prepared Enzyme- Conjugate: 1 week at 2 °C to 8 °C in a sealed container.*

Example:

If the whole plate is used, dilute 1.2 mL *Enzyme Conjugate* with 10.8 mL *Conjugate Diluent* to a total volume of 12 mL. If the whole plate is not used at once prepare the required quantity of Enzyme Con- jugate by mixing 100 µL of *Enzyme Conjugate 10X conc.* with 0.9 mL of *Conjugate Diluent* per strip (see table below):

No. of strips	<i>Enzyme Conjugate 10X conc.</i> (µL)	<i>Conjugate Diluent</i> (mL)
1	100	0.9
2	200	1.8
3	300	2.7
4	400	3.6
5	500	4.5
6	600	5.4
7	700	6.3
8	800	7.2
9	900	8.1
10	1000	9.0
11	1100	9.9
12	1200	10.8

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, IBL-America has to be informed in writ- ing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SAMPLE COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay. Do not use haemolytic, icteric or lipaemic samples. *Please note:* Samples containing sodium azide should not be used in the assay.

5.1 Sample Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Subjects receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Sample Storage and Preparation

Samples should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Samples held for a longer time (up to 12 months) should be frozen at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Sample Dilution

If in an initial assay, a sample is found to contain more than the highest standard, the samples can be diluted with *Sample Diluent* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) dilution 1:10: 10 µL sample + 90 µL *Sample Diluent* (mix thoroughly)
- b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Pipette **20 µL** of each *Standard*, *Control* and samples with new disposable tips into appropriate wells.
3. Add **100 µL** freshly diluted *Enzyme Conjugate* (see "Reagent Preparation") into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **120 minutes** at room temperature.
5. Wash the wells as follows:
If the wash step is performed manually:
 Briskly shake out the contents of the wells.
 Rinse the wells **5 times** with **300 µL** diluted *Wash Solution* per well.
If an automated plate washer is used:
 Rinse the wells **5 times** with **400 µL** diluted *Wash Solution* per well.
At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplet.
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
9. Measure the optical density of the solution in each well at 450 nm filter (reading) and at 620 nm to 630 nm (background subtraction, recommended).
It is recommended that the wells be read within 10 minutes after adding the *Stop Solution*.

6.3 Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Manual method: Using linear graph paper, plot the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.)
Other data reduction functions may give slightly different results.
5. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 U/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

7 QUALITY CONTROL

Good laboratory practice requires that controls be included with each run. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The use of control samples is advised to assure the day to day validity of results. The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 0.60 U/mL – 100 U/mL.

8.2 Specificity of Antibodies (Cross Reactivity)

No cross reactivity was observed with related proteins.

8.3 Sensitivity (LoB, LoD, LoQ)

The Limit of Blank (LoB) is 0.44 U/mL.

The Limit of Detection (LoD) is 0.60 U/mL.

The Limit of Quantification (LoQ) is 0.82 U/mL.

8.4 Reproducibility

8.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)	Status
Sample 1	40	3.79	4.7	passed
Sample 2	40	6.35	3.7	passed
Sample 3	40	15.89	3.0	passed
Sample 4	40	63.44	4.1	passed

8.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)	Status
Sample 1	80	3.79	10.1	passed
Sample 2	80	6.35	8.4	passed
Sample 3	80	15.89	6.1	passed
Sample 4	80	63.44	6.0	passed

8.5 Recovery

Samples have been spiked by adding CA 72-4 solutions with known concentrations in a 1:1 ratio.

The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

	Sample 1	Sample 2	Sample 3
Concentration [U/mL]	3.6	8.1	9.4
Average Recovery [%]	99.3	98.2	98.8
Range of Recovery [%]	from	92.5	88.2
	to	102.1	106.8

8.6 Linearity

	Sample 1	Sample 2	Sample 3
Concentration [U/mL]	51.0	94.0	10.0
Average Recovery [%]	91.2	108.5	97.8
Range of Recovery [%]	from	106.4	86.0
	to	99.6	112.3

9 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

9.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results. Triglycerides > 7.5 mg/mL will result in decreased values. The assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.

9.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CA 72-4 in a sample.

9.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 6,400 U/mL of CA 72-4.

10 LEGAL ASPECTS














10.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IBL-America.

10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta