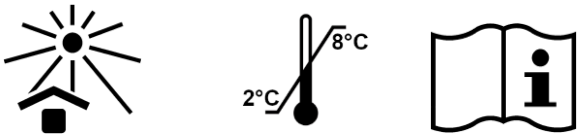
Beta-2 GP1 IgM ELISA

Instructions for Use

REF 0811FE00 Σ 12 x 8 determinations

IVD CE



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Contents

The product described here complies with the requirements of the IVD Directive 98/79/EC and transitional provisions of Article 110 of 2017/746 IVD Regulation.

1. Overview

1.1 Introduction and background

The anti-phospholipid syndrome (APS) is a systemic autoimmune disorder which can comprise clinical conditions as venous and arterial thrombosis, thrombocytopenia, myocardial infarction, recurrent spontaneous abortion and neurological complications (1, 2, 3, 4). In addition to these clinical manifestations, the persistent presence of a unique collection of autoantibodies is what defines the syndrome. These autoantibodies target specific phospholipids and phospholipid-binding proteins.

Among the phospholipids, Cardiolipin (CL) is the most common one, negatively charged and acid. beta2-glycoprotein 1 (ß2-GP1; = apolipoprotein H) has been identified as natural and essential co-antigen for CL-autoantibodies (5, 6). Besides this diagnostic significance, these antibodies cause a hypercoagulable state, associated with a tendency towards thromboses (4, 7, 8), and are believed to be directly involved in the pathogenesis of APS (9, 10). The actual mechanism of this effect however remains elusive.

The present enzyme-linked immunosorbent assay (ELISA) is intended for the quantitative or qualitative determination of IgM antibodies directed against ß2-GP1 in human serum or plasma (cf. section 7). The immobilised antigen is a highly purified preparation of human ß2-GP1. The test is fast (incubation time 30 - 30 - 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). Six calibrators allow quantitative measurements; a negative and a positive control check the assay performance.

1.2 Intended Purpose

Beta-2 GP1 IgM ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the quantitative or qualitative determination of IgM class antibodies directed against β-2-Glycoprotein 1 in human serum or plasma samples.
Its function is the aid to diagnosis of anti-phospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE).
This product is intended for manual or automated professional in vitro diagnostic use only.

2. Warnings and precautions

The test kit is intended for in vitro diagnostic use only; not for internal or external use in humans or animals. It must be executed by trained professional staff.

The kit has been tested for transport stability and can be shipped unrefrigerated for up to 3 days. Store at 2 - 8°C on arrival. In case of doubt, contact your local distributor or the manufacturer.

Do not use reagents beyond their expiration dates.

Adherence to the protocol is strongly recommended.

The sample buffer, calibrators and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The stop solution, 0,2 M sulfuric acid (H_2SO_4), is acidic and corrosive.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a manner complying with local/national regulations.

Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

The calibrators and controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.

However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Diseae Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.

3. Principle of the test

The wells of the solid phase are coated with ß2-GP1. On this surface, the following immunological reactions take place:

1st reaction: ß2-GP1-specific antibodies present in the sample bind to the immobilised antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.

2nd reaction: A second antibody, directed at human IgM antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed away from the solid phase.

3rd reaction: The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour development reflects the concentration of ß2-GP1 IgM in the sample.

4. Contents of the kit

a. Beta-2 GP1 Coated Microwell Plate

1 microwell plate, coated with ß2-GP1 and hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.

MWP	12x8

b. Sample buffer

Sample buffer, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.

BUF	SPL

c. Wash buffer

Wash buffer, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.

BUF	WASH	10x

d. Beta-2 GP1 IgM Calibrator 1-6

6 calibrators, 2,0 mL each, 0 - 3,0 - 8,0 - 18 - 45 and 100 U ß2-GP1 IgM / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.

CAL	1-6

e. Beta-2 GP1 IgM Negative and Positive Control

Negative and positive control, 2,0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

CONTROL	−

CONTROL	+

f. Beta-2 GP1 IgM 14 mL Conjugate

Anti-human IgM HRP conjugate, 14 mL, ready-to-use, green coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.

CONJ	IgM

g. Substrate

Substrate solution, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.

SUBS	TMB

h. Stop solution

Stop solution (0,2 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.

SOLN	STOP

i. Instructions for Use

Lot-specific certificate of analysis

5. Materials required but not supplied

a. Deionised or distilled water

b. Graduated cylinder, 1000 mL

c. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)

d. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)

e. Microwell plate washer (optional)

f. Microwell plate photometer fitted with a 450 nm filter

g. ELISA evaluation program (recommended)

6. Storage of the kit

Store kit at 2 - 8°C, do not freeze. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

7. Reagent and sample preparation / specimen requirements

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is **crucially important** that no cross-contamination between the reagents occurs. Use only clean pipettes and do **not pour back** residues into the original flasks.

a. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).

c. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA-, citrate- or heparin-treated plasma is suitable sample material as well.

   Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.

   Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.

   For rapid dispensing during the assay procedure, preparation of the calibrators, controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.

   If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

8. Assay procedure

8.1. Manual operation

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, **careful washing** is essential (steps a, c and e). It is **crucially important to remove the wash solution completely.** For that purpose, tap the plate firmly on

several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

a. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.

b. Dispense the calibrators (2,0 mL each, ready-to-use, gradually blue), controls (2,0 mL each, ready-to-use, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended.

   Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

c. Wash the wells 4 times as in step a.

d. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, green); 100 µL per well. Incubate the plate as in step b.

e. Repeat wash step c.

f. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colourless, black vial); 100 µL per well. Incubate the plate as in step b. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.

g. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.

h. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

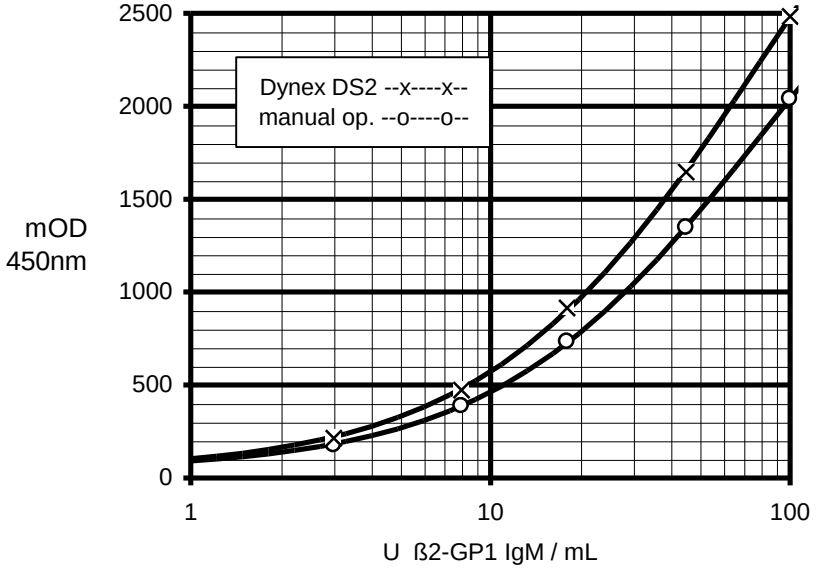
8.2. Dynex DS2 automated ELISA system

This product has been validated for use with the Dynex DS2 automated ELISA system. A description of the program flow for the assay execution and evaluation can be provided as a pdf file. The parameters of this program are merely a proposal and may need to be adapted by the operator to the requirements of the actual assay. In general terms, we have attempted to stick as close as possible to the protocol of manual operation, as above. However, due to the necessarily elevated temperature within the DS2, the substrate incubation period had to be shortened.

Section 11.8. gives a performance comparison between manual assay operation and the DS2 ELISA system.

9. Evaluation and quality control

Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.



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If no computer-supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into U ß2-GP1 IgM per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance (= cut-off). It is determined according to the following formula:

$$\text{absorbance}_{\text{borderline}} = \text{absorbance}_{\text{positive control}} \times \text{factor}$$

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with each test kit. Example:

$\text{absorbance}_{\text{positive control}}$ = 1250 mOD
factor = 0,35
$\text{absorbance}_{\text{borderline}}$ = 1250 mOD x 0,35 = 438 mOD

In order to gain an impression of how positive a particular sample is for ß2-GP1 IgM, one may calculate the ratio, according to the formula:

ratio = $absorbance_{sample}$ / $absorbance_{borderline}$

Example:

$absorbance_{borderline}$ = 438 mOD
$absorbance_{sample}$ = 1480 mOD
ratio = 1480 mOD / 438 mOD = 3,4

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

10. Interpretation of results / limitations of the procedure

Based on the measurement of a blood donor and a positive collective of sera (see below), we suggest for the assessment of patient sera:

	quantitative evaluation U ß2-GP1 IgM / mL sample	qualitative evaluation ratio
normal (negative) range	< 10,0	< 0,87
cut-off	12,0	1,00
equivocal range	10,0 - 14,4	0,87 - 1,15
positive range	> 14,4	> 1,15

These specifications are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the patient does not have an elevated level of IgM antibodies to ß2-GP1. If characteristic clinical signs of APS are nevertheless observed, IgA/IgG antibodies directed at ß2-GP1 and/or antibodies directed at CL should be determined.

A positive result should be considered as an indication for APS, as outlined in the beginning. However, it needs to be positive on at least two occasions, separated by 12 weeks, to be considered diagnostic for APS (1).

Specimens exhibiting results within the borderline range quoted above should be considered as equivocal and reported as such. It is recommended that a second sample be collected two weeks later and run in parallel with the first sample to document a possible change of antibody titer.

As with any serological test, the results should be interpreted in the light of the patient's symptoms and other diagnostic criteria.

11. Performance characteristics

11.1. Standardisation

The test is standardised with a purified serum preparation containing IgM antibodies specifically directed at ß2-GP1. This preparation is calibrated against a set of gradually positive sera, solely reserved for this purpose. The degree of sample reactivity is measured in arbitrary units (U ß2-GP1 IgM / mL) since no international standard is available.

11.2. Analytical specificity

The test allows the specific determination of human IgM antibodies directed against ß2-GP1.
Interference with anticoagulants (EDTA, Citrat, Heparin) in samples has been tested and no interference effects have been observed.

11.3. Detection limit (analytical sensitivity)

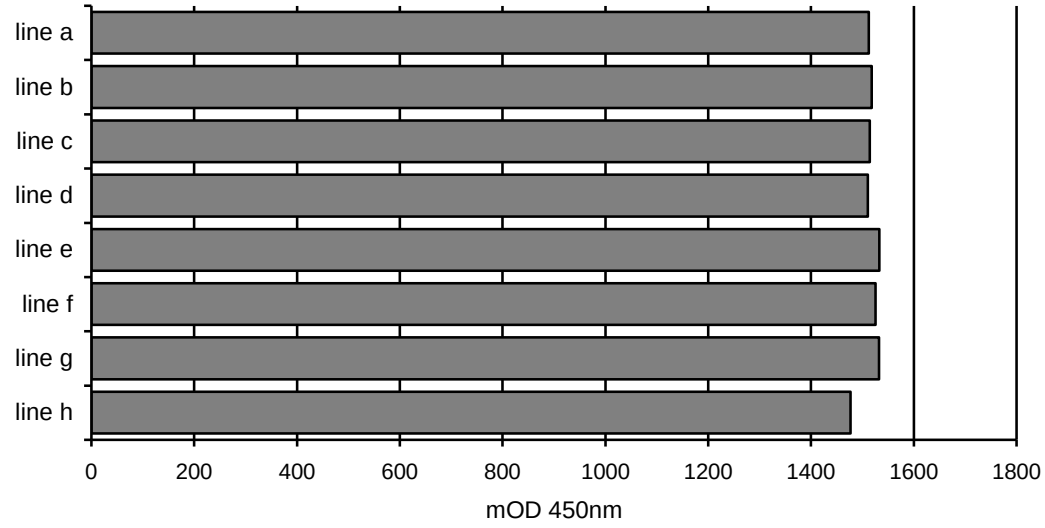
The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of sample buffer plus 3-fold standard deviation (s). It was determined as < 1 U ß2-GP1 IgM per mL sample (n = 24).

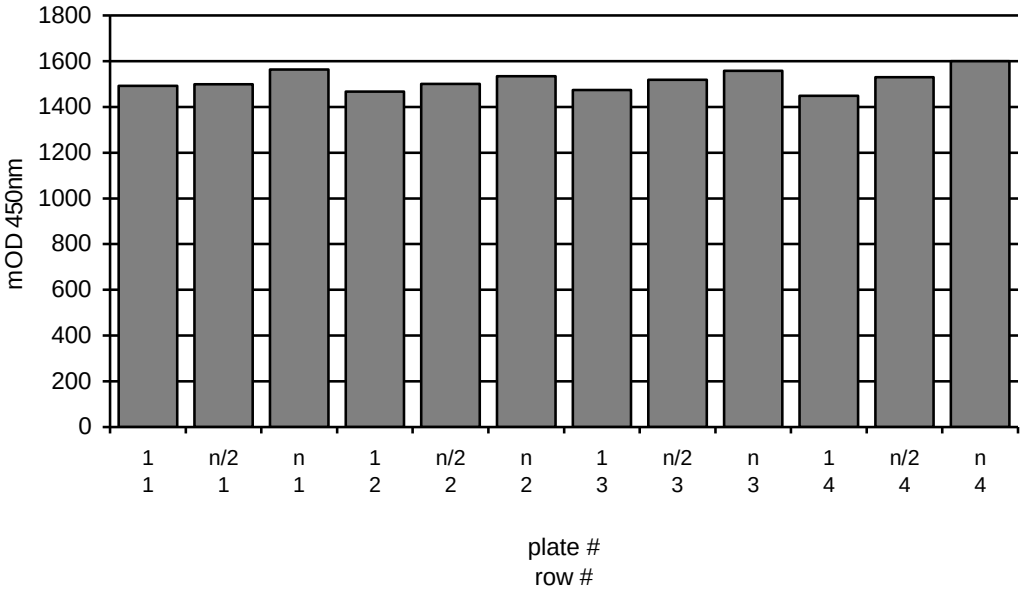
Recommended measuring range: 2 - 100 U ß2-GP1 IgM per mL sample

11.4. Homogeneity of the solid phase

Measurement of the solid phase homogeneity is a regular QC part of each production lot. This is determined by 288-fold measurement of an IgG-positive but non-saturating sample on 3 selected plates. Acceptance criterion: mOD-coefficient of variation (cv) over the plates < 8%. The figure below shows a representative excerpt (solid phase lot no. 0104S) of such an analysis.

plate row	1 1	n/2 1	n 1	1 2	n/2 2	n 2	1 3	n/2 3	n 3	1 4	n/2 4	n 4	mean	cv %
line a	1496	1505	1585	1455	1516	1519	1546	1505	1526	1413	1510	1572	1512	3,1
line b	1498	1516	1581	1465	1526	1550	1444	1516	1559	1425	1512	1628	1518	3,8
line c	1520	1517	1544	1473	1520	1533	1440	1518	1545	1434	1510	1617	1514	3,2
line d	1481	1479	1541	1449	1512	1549	1463	1534	1554	1445	1542	1580	1511	3,0
line e	1508	1547	1586	1497	1507	1545	1476	1537	1604	1441	1542	1606	1533	3,3
line f	1503	1493	1578	1480	1476	1547	1461	1528	1599	1473	1545	1622	1525	3,5
line g	1489	1501	1577	1467	1513	1558	1495	1526	1577	1504	1574	1609	1533	2,9
line h	1447	1440	1519	1451	1433	1467	1464	1487	1499	1450	1508	1560	1477	2,6
mean	1493	1500	1564	1467	1500	1534	1474	1519	1558	1448	1530	1599	**1515**	
cv %	1,5	2,1	1,6	1,1	2,1	1,9	2,3	1,1	2,3	2,0	1,6	1,6		**3,3**





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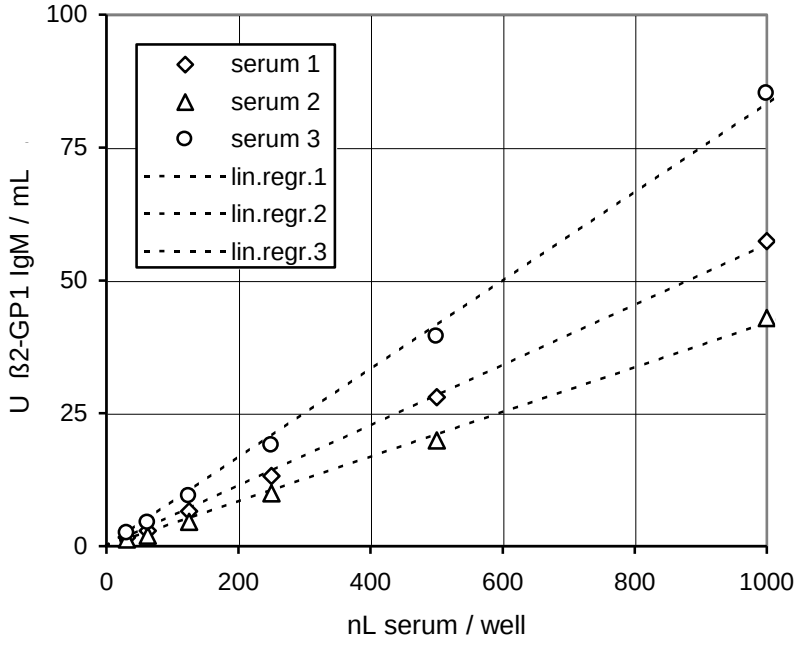
11.5. Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor > 0,98. A typical result is depicted below.



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11.6. Precision

For the assessment of the test precision, the variability of results under the following conditions was determined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	mean U/mL	variability (cv, %) intra-assay	inter-assay
1	7,9	6,1	6,9
2	18,7	4,4	6,7
3	47,7	3,8	6,4

b. Operator to operator variability (n = 12)

sample	mean U/mL	variability (cv, %)
1	8,4	2,9
2	19,9	2,5
3	44,8	2,7

c. Variability between 2 kit lots (n = 6)

sample	mean U/mL	variability (cv, %)
1	8,4	2,5
2	19,5	4,5
3	48,0	4,9

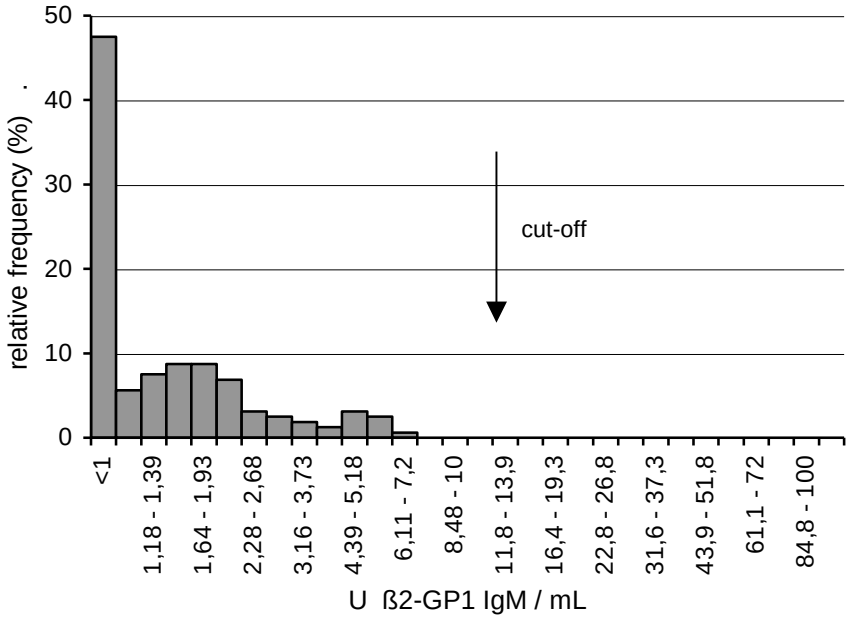
11.7. Frequency distribution of ß2-GP1 IgM

This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of positive sera according to a CE-compliant reference ELISA. The following distribution of the analyte was observed:

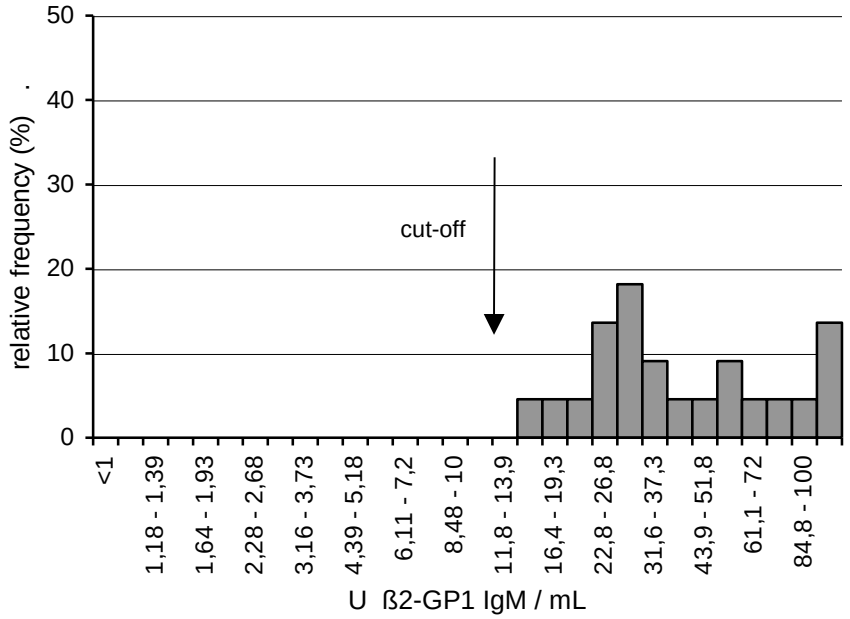
blood donor sera			**positive sera**		
n:	160		n:	22	
mean:	1,5	U/mL	mean:	59,5	U/mL
mean + s:	2,7	U/mL	mean - s:	4,4	U/mL
mean + 2s:	4,0	U/mL	mean - 2s:	< 0	U/mL
median:	1,1	U/mL	median:	35,6	U/mL
95th percentile:	4,8	U/mL	5th percentile:	16,9	U/mL

ROC-analysis of these data was used to determine the cut-off as 12,0 U ß2-GP1 IgM / mL (11). The data presented here suggest a diagnostic specificity and sensitivity of the ELISA of nearly 100 % for both parameters. These values apply for the measured sera only; other collectives may yield different results. In view of the low number of positive sera, particular caution is required when interpreting test sensitivity.

blood donor sera



positive sera



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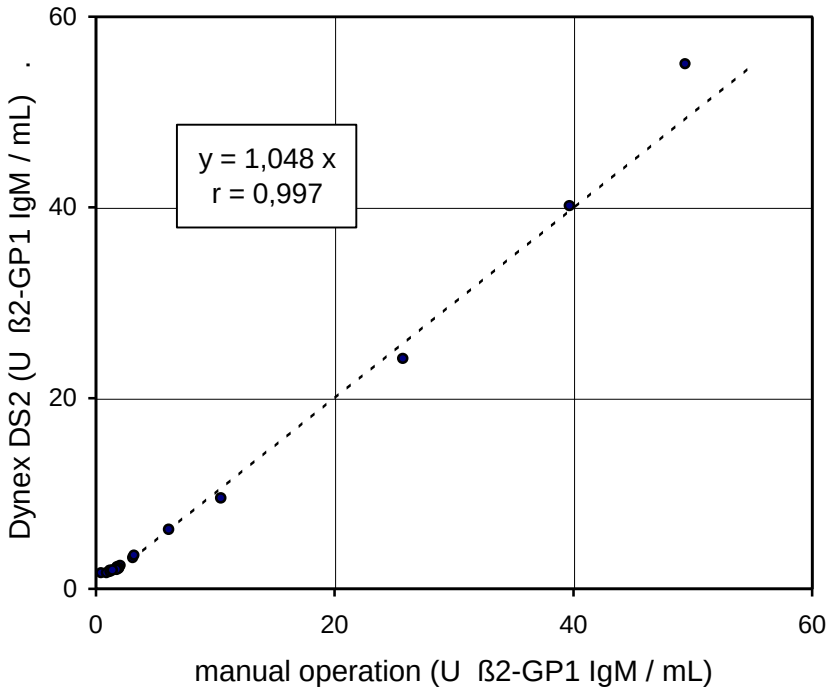
11.8. Manual operation vs. Dynex DS2 automated ELISA system

Variability: Using specimen of one and the same kit lot, the variability of assay results were compared between manual operation and the Dynex DS2 automated ELISA system:

	manual operation	Dynex DS2
intra-assay variability (n = 16)	mean cv = 1,6 %	mean cv = 2,3 %
inter-assay variability (n = 48)	mean cv = 4,7 %	mean cv = 3,1 %

Standard curve: depicted in section 9

Correlation:



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12. Declaration

Steffens Biotechnische Analysen GmbH (SBA) guarantees that the product delivered has been thoroughly tested to ensure that its properties specified herein are fulfilled. No further warranties are given.

The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case SBA disclaims all warranties whether expressed, implied or statutory. Moreover, SBA accepts no liability for any damage, whether direct, indirect or consequential, which results from inappropriate use or storage of the product.

13. Symbols

Catalogue number

Batch code

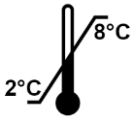
Unique Device Identification

Contains sufficient for <n> tests

In Vitro diagnostic medical device

Conformité Européenne

Keep away from sunlight



Store at 2 - 8°C

Use-by Date

Consult “Instructions for Use”

Caution

Biological risk

Manufacturer

Distributor

14. References

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15. Summary flow chart

a. Dilute the samples 1/100 in sample buffer (100 mL, ready-to-use, orange) and mix.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with water and mix.

c. Wash the wells once with 350 µL wash buffer each. Dispense 100 µL of the calibrators (2,0 mL each, ready-to-use, gradually blue) and controls (2,0 mL each, ready-to-use, green and red) and of the diluted samples into the wells of the solid phase. Duplicate measurements are recommended. Incubate for 30 minutes at room temperature (23 ± 3°C).

d. Wash the wells 4 times with 350 µL wash buffer each.

e. Dispense 100 µL of the conjugate (14 mL, ready-to-use, green) into the wells. Incubate as in step c.

f. Repeat washing step d.

g. Dispense 100 µL of the substrate solution (14 mL, ready-to-use, black vial) per well. Incubate as in step c. Then, add 100 µL stop solution (14 mL, ready-to-use, colourless) per well and agitate the plate briefly.

h. Immediately measure the absorbance at 450 nm.

i. Quantitative evaluation: determine the standard curve and, using this curve, transform the absorbance of the samples into their respective antibody concentration (U/mL).

j. Qualitative evaluation: determine the borderline absorbance by multiplying the absorbance of the positive control with the factor shown in the certificate of analysis. Then, calculate the ratio of the samples by dividing their absorbance by the borderline absorbance.