



## **Diacordon™ ELISA**

### **ALX-DIC-E-051-KI01**

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#### ***NORTH AMERICA***

**AXXORA, LLC**  
6181 Cornerstone Court East  
Suite 103  
San Diego, CA 92121-4727  
Phone: (858) 550-8828  
Fax: (858) 550-8825  
E-mail: [axxora-usa@axxora.com](mailto:axxora-usa@axxora.com)

#### **SWITZERLAND/REST OF THE WORLD**

**ALEXIS CORPORATION**  
Industriestrasse 17, Postfach  
CH-4415 Lausen / Switzerland  
Phone: +41 61 926 89 89  
Fax: +41 61 926 89 79  
E-mail: [alexis-ch@alexis-corp.com](mailto:alexis-ch@alexis-corp.com)

#### **GERMANY**

**AXXORA DEUTSCHLAND GmbH**  
Gallusstrasse 10  
DE-35305 Grünberg  
Phone: (06401) 90077  
Fax: (06401) 90078  
E-mail: [axxora-de@axxora.com](mailto:axxora-de@axxora.com)

#### **UK & IRELAND**

**AXXORA (UK) LTD.**  
P.O. Box 6757  
Bingham, Nottingham NG13 8LS  
Phone: +44 1949 836111  
Fax: +44 1949 836222  
E-mail: [axxora-uk@axxora.com](mailto:axxora-uk@axxora.com)

**For laboratory use only. Not for human or diagnostic use.**

## Intended Use

The **Diacordon® Glycogen Phosphorylase Isoenzyme BB (GP-BB) ELISA** is an *in-vitro*-diagnostic device for the quantitative determination of GP-BB in undiluted human heparinized plasma samples.

## Principle Of The Test

Diacordon® Glycogen Phosphorylase Isoenzyme BB (GP-BB)-ELISA is a fast enzymometric one-step assay based on monoclonal antibodies (mouse) with high affinity.

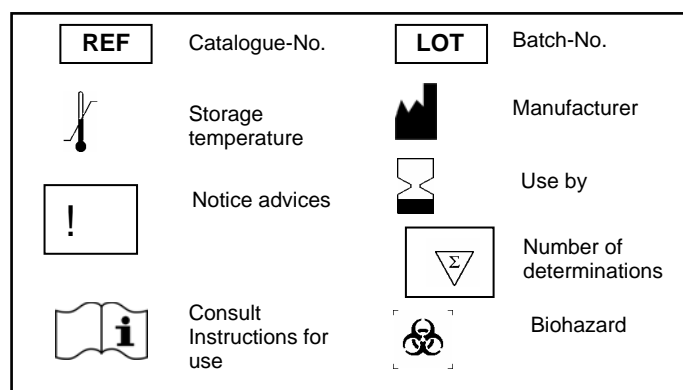
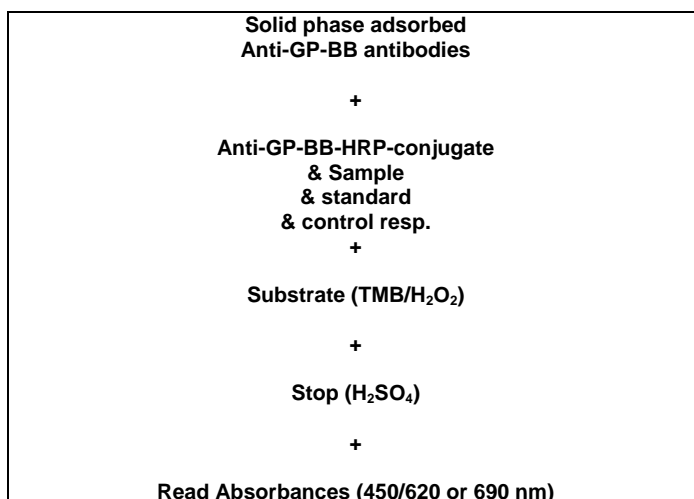
Specimens, standards and control resp. are dispensed simultaneously with the horseradish peroxidase (HRP) labelled anti-GP-BB-antibodies into the wells of a microtitration plate coated with anti-GP-BB-antibodies.

After an incubation time of 30 min at 37 °C unbound components are removed by a washing step.

HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 15 min reaction time into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow.

The resulting absorbances are measured at 450 nm (reference filter 620 or 690 nm).

A standard curve is created from the absorbances of the different GP-BB standard concentrations. The absorbances of the unknown samples can be transformed into the corresponding GP-BB concentrations by reading from the standard curve.



## Preparation And Storage Of Samples

### Collection and storage

Blood samples should be collected with appropriate cuvettes using only heparin for anticoagulation.

Samples should be centrifuged immediately (not later than 30 minutes) after collection at 3000 g – 4000 g for 10 minutes.

ELISA testing of the samples should be started within one hour after blood collection.

In case of retrospective testing samples should be frozen at – 20 °C or less within one hour after blood collection. When testing frozen samples, make sure that they have warmed to room temperature after thawing and mix them thoroughly before starting the assay. Once thawed, samples should be tested within one hour. Repeated freezing and thawing of samples should be avoided.

### Preparation

Heparinized human plasma samples should be tested in the GP-BB ELISA without further dilution.

### Test Components For 96 Wells

<b>1</b> <b>WELLS</b>	<b>Microtitration plate</b> 12 single breakable 8-well strips (in all 96 wells) coated with monoclonal anti-GP-BB-antibodies (mouse)	1 vacuum-sealed with desiccant
<b>2</b> <b>WASHBUF 10X</b>	<b>Wash buffer, 10-fold</b> for 1000 ml solution Tris-buffer (0.05 mol/l) pH 7.4 ± 0.1; contains 0.01 % (w/v) thiomersal	2 x 50 ml concentrate white cap
<b>3</b> <b>STD 1-5</b>	<b>Standard S1 – S5</b> S1= 100 ng/ml; S2 = 50 ng/ml; S3 = 20 ng/ml; S4 = 8 ng/ml S5 = 3 ng/ml Contains 1.0 % (v/v) kathon	5 x 1.0 ml ready to use white cap
<b>4</b> <b>CONTROL +</b>	<b>Positive control</b> Positive control = 35 ng/ml	1.0 ml ready to use green cap
<b>5</b> <b>CONJ HRP</b>	<b>HRP-conjugate</b> HRP-labelled, monoclonal anti-GP-BB-antibodies (mouse) contains 1.0 % (v/v) kathon	12 ml ready to use red cap
<b>6</b> <b>SUBSTR TMB</b>	<b>Substrate</b> 3,3',5,5'-Tetramethylbenzidine and 0.03 mol/l hydrogen peroxide contains 0.008 % kathon	15 ml ready to use blue cap
<b>7</b> <b>STOP</b>	<b>Stop solution</b> 0.25 mol/l sulphuric acid	15 ml ready to use yellow cap
<b>8</b>	<b>Plate sealer</b>	1
<b>9</b>	<b>Instructions for use</b>	1

### Materials Required But Not Provided

- adjustable one channel micropipettes (0.100-1.000 ml and 0.010-0.100 ml and pipette tips
- adjustable 8 channel micropipette (0.050-0.200 ml) and pipette tips
- Reagent container for 8 channel pipette
- microplate washer (automatic or hand wash head)
- microplate reader with optical filters for 450 nm and 620 or 690nm
- distilled or de-ionized water
- glassware
- stop-watch

## Preparation And Storage Of Reagents

### Kit size and expiry

One kit enables quantification of GP-BB in a maximum of 42 plasma samples when samples, standards and control are run in duplicate.

The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.

Upon receipt, all test components have to be kept at 2...8 °C, preferably in the original kit box.

After opening, all kit components are stable for at least 2 months, provided proper storage.

### Reagent preparation

Allow all components to reach room temperature prior to use in the assay.

The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the 10fold concentrated wash buffer 1 + 9 with distilled or de-ionized water.

#### *For Example:*

10 ml wash buffer concentrate + 90 ml distilled water.

This ready to use wash buffer solution is stable for at least 30 days when stored at 2...8 °C.

### Assay Procedure

- Standards, control and samples should be run in duplicate.
- Create a new reference curve for each test run.
- Avoid any time shift during dispensing of reagents and samples, independent of their position in the microtitration plate.
- After the first incubation of 30 min remove the samples from the wells with the same velocity and in the same order as initially dispensing them to the wells when starting the incubation.
- If larger numbers of samples have to be investigated, pre-dispensing of the samples into the wells of an uncoated microplate and subsequent transfer of the pre-dispensed samples into the coated microplate of the assay with an 8 channel pipette will be recommended to minimize time delay.
- Use the data sheet provided with the kit. Adhere to the prescribed layout regarding positioning of the standards and the control.
- Make sure that the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle.
- Avoid light exposure of the TMB substrate solution.

## Working steps

1. Warm all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense  
**100 µl CONJ POD (5)** to all wells and add
3. **100 µl undiluted samples,**  
**100 µl Standards (3),**  
**100 µl CONTROL + positive control (4)** to the intended wells  
Mix the reagents thoroughly by drawing up and expelling the fluid twice with the pipette.
4. Cover plate and incubate for **30 min** at 37 °C without shaking.
5. Decant, then wash each well **5x** with **300 µl** wash solution (diluted from (2)) and tap dry onto absorbent paper after the final washing step.
6. Dispense  
**200 µl SUBSTR TMB (6).**
7. Incubate for **15 √ 1 min** at room temperature protected from light.
8. Dispense  
**100 µl STOP (7),** mix gently.
9. Read absorbances at **450 nm** (reference filter 620 or 690 nm) with a microplate reader within 30 min after reaction stop.

## Result Interpretation

Calculate the mean absorbances of standards, control and samples. Create a reference curve from the mean absorbances of the standards S1-S5 (y-axis) and their corresponding GP-BB concentrations (x-axis). Determine the GP-BB concentrations of the unknown samples by referring their mean absorbances to the reference curve.

## Reference Values

## Cut-Off

The GP-BB concentration in plasma samples of European normal healthy blood donors in general not exceeds 10 ng/ml. However a GP-BB concentration of more than 10 ng/ml as isolated laboratory parameter is not equivalent to a diagnosis. The final result interpretation has to consider clinical findings as well.

## Diacordon® Glycogen Phosphorylase Isoenzyme BB (GP-BB)-ELISA

Negative	< 10 ng/ml
Positive	> 10 ng/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

## Test validity

The test run is valid if:

- the mean absorbance of standard 5 (3 ng/ml) is  $\leq 0.50$
- the mean absorbance of standard 1 (100 ng/ml) is  $\geq 1.50$
- the mean concentration of the control is  $> 30$  ng/ml and  $< 45$  ng/ml

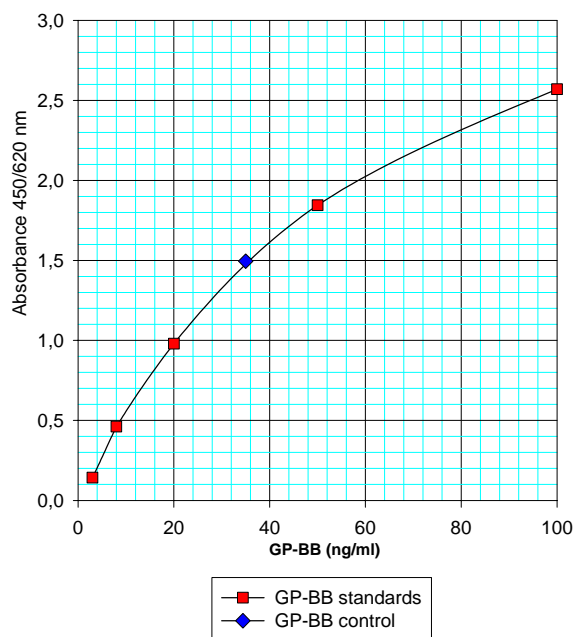
If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

## Limitations of the procedure

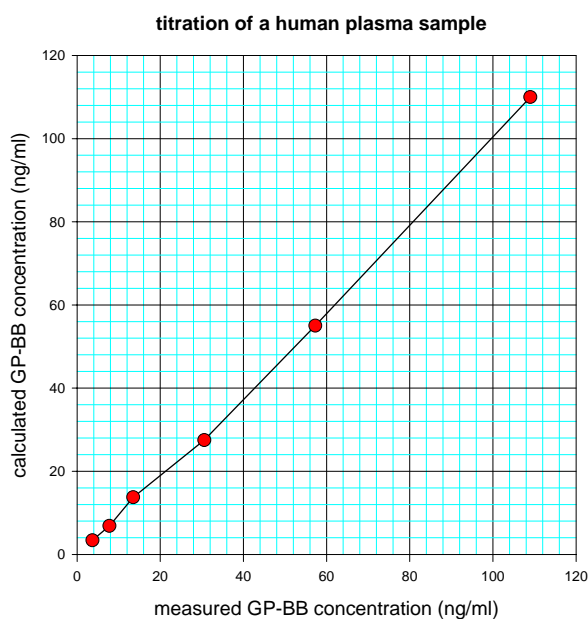
False results may be caused by cross contamination of the kit reagents and samples or by bacterial or fungal contaminations of reagents and/or samples, incorrect washing and incorrect incubation times. High concentrations of human anti-mouse antibodies (HAMA) may cause false results and have to be considered in case of clinically non plausible high GP-BB values. Because of the limited stability of glycogen phosphorylase isoenzyme BB the assay has to be run within one hour after blood collection or thawing of frozen samples, otherwise false negative results may occur. Using other substances than heparin for blood anticoagulation may cause false results and should be avoided.

## Performance Characteristics

### Typical Calibration curve in linear presentation



## Dilution linearity



## Precision

Intra-assay coefficient of variation (CV) of absorbances in the *Diacordon*<sup>®</sup> GP-BB-ELISA from 12fold determinations of samples:

Sample	Mean absorbance	Standard deviation	CV (%)
1	2.280	0.038	1.67
2	2.010	0.084	4.20
3	1.600	0.051	3.20
4	1.056	0.052	4.95
5	0.921	0.044	4.74
6	0.569	0.036	6.33
7	0.486	0.022	4.55
8	0.295	0.020	6.85
9	0.258	0.016	6.27
10	0.130	0.008	6.39

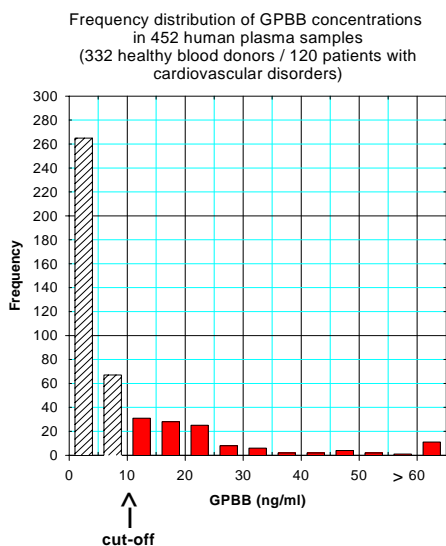
Intra-assay coefficient of variation (CV) of concentrations in the *Diacordon*<sup>®</sup> GP-BB-ELISA from 12fold determinations of samples:

Sample	Mean concentration (ng/ml)	Standard deviation	CV (%)
1	100.5	2.43	2.41
2	72.5	1.80	2.49
3	50.2	1.34	2.66
4	43.0	0.71	1.64
5	25.1	1.57	6.27
6	21.9	0.52	2.37
7	12.5	0.62	4.95
8	11.4	0.58	5.06
9	5.0	0.17	3.32

Inter-assay coefficient of variation (CV) of concentrations in the *Diacordon*<sup>®</sup> GP-BB-ELISA from 16 different test runs (samples run in duplicate)

Sample	Mean concentration (ng/ml)	Standard deviation	CV (%)
1	108.7	6.70	6.20
2	40.9	2.50	6.04
3	21.0	0.95	4.52
4	10.5	0.93	8.83
5	6.2	0.29	4.75
6	43.3	1.75	4.05
7	27.8	1.30	4.68
8	22.6	0.90	4.12
9	12.2	1.26	10.3
10	5.8	0.20	3.45

## Frequency distribution



## Clinical evaluation

Blood plasma samples of the following patient groups were investigated for glycogen phosphorylase isoenzyme BB (GP-BB) in comparison to the "cardiac markers" myocardial troponin T (CTNT), creatin kinase isoenzyme MB (CKMB) and myoglobin.

### Patients with acute cardiovascular disorders:

Percutane transluminale coronary angioplasty (PTCA): n = 24  
 Non stable angina pectoris (IAP): n = 24  
 Acute myocardial infarction (AMI): n = 37

### Control group:

Healthy blood donors: n = 50  
 Patients with coronary bypass operation: n = 16  
 Patients with elevated creatin kinase concentration: n = 7  
 Patients with cardiac insufficiency: n = 10  
 Dialysis patients: n = 18  
 Patients with hepatitis: n = 11  
 Patients with liver cirrhosis: n = 10  
 Patients after reanimation: n = 3

Table 1 summarizes the results gained from the investigation of patients suffering from acute myocardial infarction.

Table 1:

Hours after onset of chest pain/ Patients with acute myocardial infarction (AMI)	cTNT		CKMB		Myoglobin		GP-BB	
	Cut-Off 0.1 ng/ml		Cut-Off 6.1 ng/ml		Cut-Off 70 ng/ml		Cut-Off 10 ng/ml	
	S E N S. %	S P E C. %	S E N S. %	S P E C. %	S E N S. %	S P E C. %	S E N S. %	S P E C. %
1 h/ n=12	54.5	100	63.6	90.0	90.9	90.0	91.7	98.0
2 h/ n=22	57.1	100	71.4	90.0	85.7	90.0	90.9	98.0
3 h/ n=23	68.2	100	81.8	90.0	95.2	90.0	95.7	98.0
4 h/ n=24	69.6	100	91.3	90.0	95.7	90.0	91.7	98.0

In comparison to the cardiac markers cTNT, CKMB and myoglobin GP-BB showed a higher sensitivity ranging between 90.9% and 95.7% and a specificity of 98.0% within the first 4 hours after onset of chest pain in patients with acute myocardial infarction.

Eighty percent of patients with non stable angina pectoris were characterized by GP-BB concentrations exceeding the cut-off value of 10 ng/ml between 0 and 36 hours after onset of chest pain.

Whereas the plasma GP-BB concentrations in the healthy blood donor group were generally determined below this cut-off value, a few patients with different cardiac and non cardiac diseases showed moderately elevated GP-BB levels.

Enhanced GP-BB values caused by cardiac ischemia may be assumed for bypass patients, patients after reanimation and patients suffering from cardiac insufficiency.

Cases of clinically silent cardiac ischemia are reported by enhanced cTNT values for dialysis patients in clinical trials elsewhere.

The specificity was determined by investigation of 50 healthy blood donors and calculation of the optimum cut-off value by ROC-analysis.

## References

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- Peetz D, Schütt S, Sucke B, Faldum A, Wandel E, Hafner G, Lackner K: Prognostic Value of Troponin T, Troponin I, and CK-MBmass in Patients with Chronic Renal Failure. *Medizinische Klinik* 2003; 98: 188-92 (Nr. 4), Urban & Vogel, München
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- Rabitzsch G, Mair J, Lechleitner P, Noll F, Hofmann V, Krause EG, Dienstl F, Puschendorf B: Isoenzyme BB of glycogen phosphorylase b and myocardial infarction. *Lancet*. 1993 Apr 17;341(8851):1032-3.

## Incubation Scheme

### *Diacordon*<sup>®</sup> GP-BB-ELISA (E-051)



**100 µl**      **CONJ POD (5)**



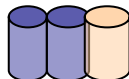
+



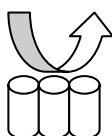
**100 µl**      **undiluted human heparin plasma samples**

**100 µl**      **Standard S1 – S5 (3)**

**100 µl**      **CONTROL + (4) positive control**



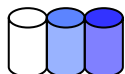
**30 min**      incubation at 37 °C



**5 X Wash**      with wash buffer



**200 µl**      **SUBSTR TMB (6)**



**15 min**      incubation at room temperature, protected from light



**100 µl**      **STOP (7)**



**Read**      **450/620 nm**

## Common Advices and Precautions

**This kit is for *in-vitro* use only.** Follow the working instructions carefully. The kit should be performed by trained technical staff only.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots.

Do not use reagents from other manufacturers.

Avoid any time shift during dispensing of samples and reagents.

All reagents should be kept at 2...8 °C and warmed to room temperature before testing.

Some of the reagents contain small amounts of thimerosal (< 0.1 % w/v) and kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes. In case of contact rinse with plenty of water.

Handle all components and all patient samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always wear laboratory coat and use protective gloves and protective goggles,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

**Data Sheet**

**Diacordon® GP-BB-ELISA (E-051)**

**Operator:**

**Lot-No.:**

**Date:**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	<b>S1</b>	<b>S1</b>	<b>PS3</b>	<b>PS3</b>								
<b>B</b>	<b>S2</b>	<b>S2</b>	<b>PS...</b>	<b>PS...</b>								
<b>C</b>	<b>S3</b>	<b>S3</b>										
<b>D</b>	<b>S4</b>	<b>S4</b>										
<b>E</b>	<b>S5</b>	<b>S5</b>										
<b>F</b>	<b>Contr</b>	<b>Contr</b>										
<b>G</b>	<b>PS1</b>	<b>PS1</b>										
<b>H</b>	<b>PS2</b>	<b>PS2</b>										

**S1 – S5** = Standard 1 – 5

**Contr** = Positive control

**PS 1 – PS ...** = Human plasma samples

**Note:**

**Standards, control and samples should be run in duplicate.**

**A new standard curve has to be created for every test run.**