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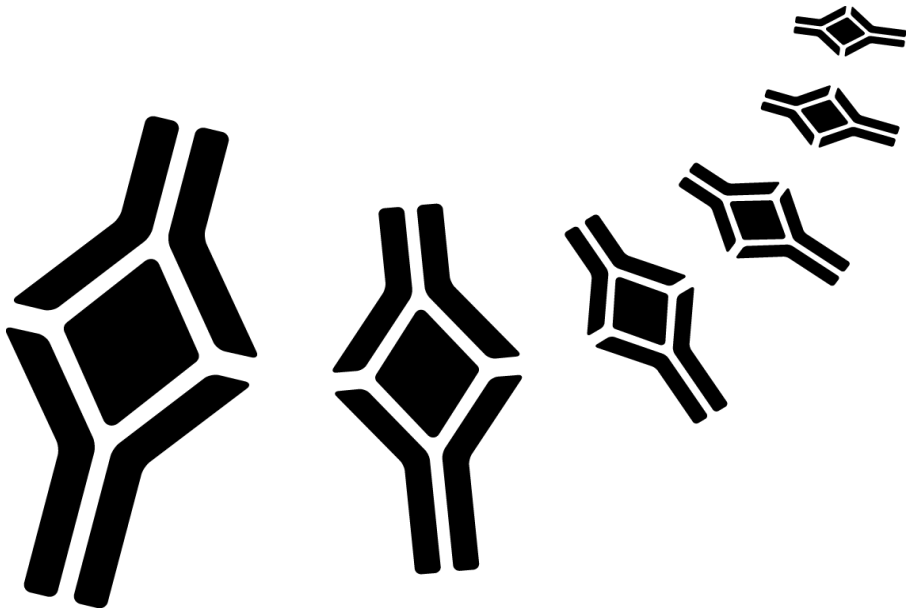
and Diagnostic Products



HUMAN FREE β-CHORIONIC GONADOTROPIN (β -HCG) ELISA

Product Data Sheet

Cat. No.: RIS0011R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RIS0011R Human Free β-Chorionic Gonadotropin (β-hCG) ELISA is an enzyme immunoassay for the measurement of free beta subunit of human chorionic gonadotropin (free β-hCG) in serum.
It is intended for research use only.

2. STORAGE, EXPIRATION

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

3. INTRODUCTION

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit.

The measurement of free β-hCG in the first trimester of pregnancy has been reported as a useful marker in antenatal screening for Down Syndrome and other fetal aneuploidies. Increased free β-hCG values in combination with maternal age, the measurement of PAPP-A and the ultrasonic determination of nuchal translucency (NT) in pregnancy weeks 11 to 14 may detect up to 90 % of pregnancies with Down syndrom (reference 15).

The Biovendor free β-hCG ELISA may be used for the risk assessment of Down´s syndrom (trisomy 21) in the first trimester of pregnancy. For the risk assessment of trisomy 21 and other fetal aneuploidies free beta hCG should always be measured in combination with other analytes (for example PAPP-A and NT, see above) and a special software for the risk assessment of trisomy 21.
According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits.

4. TEST PRINCIPLE

The Biovendor Free β-hCG 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 directed towards a unique antigenic site on a Free β-hCG molecule. An aliquot of patient sample containing endogenous Free β-hCG is incubated in the coated well with enzyme conjugate, which is an anti-β-hCG antibody [rabbit] conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of Free β-hCG in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Free β-hCG in the patient sample.

5. PRECAUTIONS

For research use only

1. 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.
2. 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.
3. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
17. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
18. 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.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from Biovendor.

6. TECHNICAL HINTS

All reagents and specimens 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.

7. REAGENT SUPPLIED

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-β-hCG antibody (monoclonal).

2. **Standard (Standard 0-5)**, 6 vials (lyophilized), 1.0 mL;
   Concentrations: 0 – 10.0 – 25.0 – 50.0 – 100.0 – 200.0 ng/mL;
   The concentrations of the Biovendor Free β-hCG Kit standards match the WHO Reference Reagent Human Chorionic Gonadotrophin, Beta Subunit (Purified) (NIBSC code: 99/650)
   See „Preparation of Reagents“.
   Contain non-mercury preservative.

3. **Control (Low and high)**, 2 vial (lyophilized), 1.0 mL,
   see „ Preparation of Reagents “
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contain non-mercury preservative.

4. **Zero Buffer**, 1 vial, 14 mL, ready to use,
   Contains non-mercury preservative.

5. **Enzyme Conjugate**, 1 vial, 18 mL, ready to use,
   Anti β-hCG antibody conjugated to horseradish peroxidase;

6. **Substrate Solution**, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).

7. **Stop Solution**, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

8. **Wash Solution**, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

Note: Additional Zero Buffer for sample dilution is available upon request.

8. MATERIAL REQUIRED BUT NOT SUPPLIED

1. A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the Biovendor Instruments Microtiter Plate Reader).
2. Calibrated variable precision micropipettes.
3. An incubator suitable for incubation 37 °C
4. Absorbent paper.
5. Distilled or Deionized water
6. Timer (60 min. range).
7. Linear graph paper or software for data reduction

9. PREPARATION OF REAGENTS

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

›› Standards

Reconstitute the lyophilized contents of the standard vials with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix several times before use.
Note: The reconstituted standards are stable for up to 30 days at 2 °C - 8 °C.
For longer storage freeze at -20 °C

›› Controls

Reconstitute the lyophilized content of the control vials with 1.0 mL distilled water and let stand for 10 minutes in minimum. Mix several times before use.
Note: The reconstituted controls are stable for up to 30 days at 2 °C - 8 °C.
For longer storage freeze at -20 °C

›› Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

›› 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 Material Safety Data Sheets (see chapter 13).

›› Damaged Test Kits

In case of any severe damage to the test kit or components, Biovendor has to be informed in writing, 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.

10. ASSAY PROCEDURE

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 50 µL of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense 100 µL *Zero Buffer* into each well.
   Thoroughly mix for 30 seconds. It is important to have a complete mixing in this step.
4. Incubate for 30 minutes at 37 °C.
5. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Dispense 150 µL *Enzyme Conjugate* into each well.
7. Incubate for 30 minutes at 37 °C.
8. Briskly shake out the contents of the wells.
   Rinse the wells 5 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add 100 µL of *Substrate Solution* to each well.
10. Incubate for 20 minutes at room temperature.
11. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well. It is important to make sure that all the blue color changes to yellow color completely.
12. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.
    It is recommended that the wells be read within 15 minutes after adding the *Stop Solution*.

11. CALCULATIONS

Calculate the average absorbance values for each set of standards, controls and patient samples. Using semi-logarithmic graph paper, construct a standard curve by plotting 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.
Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as such. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve
The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard		Optical Units (450 nm)
Standard 0	(0 ng/mL)	0.02
Standard 1	(10.0 ng/mL)	0.22
Standard 2	(25.0 ng/mL)	0.46
Standard 3	(50.0 ng/mL)	0.81
Standard 4	(100.0 ng/mL)	1.28
Standard 5	(200.0 ng/mL)	1.97

12. EXPECTED NORMAL VALUES

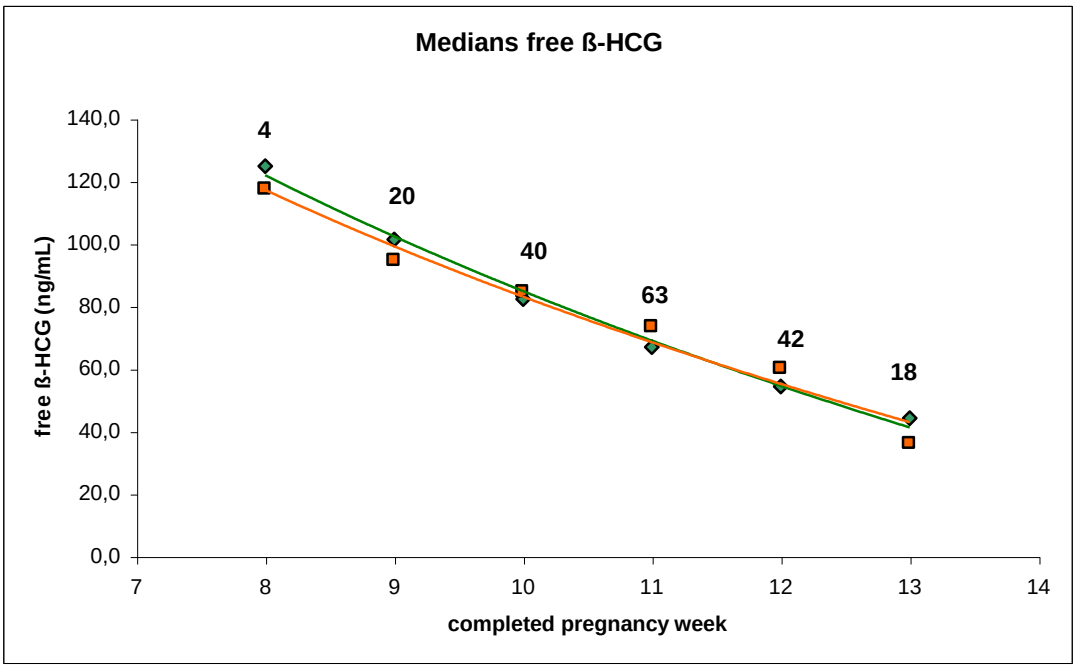
It is strongly recommended that each laboratory should determine its own normal and abnormal values.
The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

- **Free β-hCG Subunit Levels in Normal Pregnancy**

187 samples of pregnant women in the 1st trimester have been measured with the Biovendor Free β-hCG ELISA.
Using a semi-logarithmic plot the following regression equation was found:

Median free β-hCG = EXP (6.579 – 0.0297 * gestation day).

In the following diagram and table the medians for **completed pregnancy weeks** 8 to 13 have been calculated). For comparison the medians were also determined manually (Median of week).



Week of pregnancy	Day of gestation	Median from regression equation [ng/mL]	Median of week [ng/mL]
8	59	124.8	117.4
9	66	101.4	94.6
10	73	82.3	84.6
11	80	66.9	73.4
12	87	54.3	60.1
13	94	44.1	36.0

Population and laboratory differences may lead to slightly different medians. Each laboratory should therefore determine and continuously update its own medians from its own patient collective. The regression equations and values in the table should be used as a guideline only. The calculation of medians and/or regression functions for the calculation of medians from own patient data bases should be performed with the applied trisomy 21 risk calculation software. Medians determined for the Biovendor free β-hCG ELISA can not be used with assays of other manufacturers. Medians determined for free β-hCG assays of other manufacturers can not be used with the Biovendor free β-hCG ELISA.

- **Use for Down Syndrom Screening**

For risk calculation in prenatal screening free β-hCG concentrations are indicated as MOM (multiple of medians, MOM = Measured Concentration (free β-hCG) / Median free β-hCG).
In Down syndrom pregnancies the median of MOMs for free β-hCG are increasing during the first and second trimester (reference 16, details see table).

Completed week of pregnancy	10	11	12	13	14-20
Median of MOM in pregnancies with Down Syndrom	1,62	1,94	2,19	2,48	2,66

Data from reference 16

For risk calculation of trisomy 21 not only free β-hCG but also other parameters like PAPP-A and nuchal translucency (NT) for the 1st trimester and/or AFP, free Estriol and hCG for the 2nd trimester have to be determined.
The use of these parameters for risk calculation of trisomy 21 requires a special software. **According to the IVD Directive (98/79/EC) both software and kits for the additional analytes must be suitable for trisomy 21 screening and CE-certified by a notified body, indicated by the identification number of the notified body on the CE-mark on software and kits. The software must allow the calculation of medians from own patient measurements.**
It is imperative to take into consideration additional factors, e.g. age of the woman, weight, ethnic group and smoker/non-smoker. **An underestimation of the gestation age can lead to a falsely high calculated risk (false positive).** To reduce this source of error, it is important to **determine the gestation age as precisely as possible. Gestation age calculation from the last menstrual cycle inheres a high risk of variation. Sonographic determination of the crown-rump length (CRL) or biparietal diameter (BIP)** is recommended for the proper determination of the gestation age.
Free β-hCG measurement in the course of a prenatal screening determines only a risk for trisomy 21.
For proof of trisomy 21 genetic determinations are required.

- **Free β-hCG and Free Subunits Levels in Gestational Choriocarcinoma**

Free α and free β-subunits and hCG levels were measured in five patients with untreated gestational choriocarcinoma. The concentrations in serum are shown in the following table.

Patient Number	hCG (ng/mL)	Free α-hCG (ng/mL)	Free β-hCG (ng/mL)
1	210,000	112	8,000
2	22,195	20	1,300
3	6,840	1	232
4	36,000	44	3,900
5	4,200	2	350

The levels of free α-hCG were low, ranging from 1-112 ng/mL, whereas hCG levels ranged from 4,200 to 210,000 ng/mL (1 ng ≈ 15 mIU). In contrast, free β-hCG concentrations were found to be markedly elevated in choriocarcinoma.

- **Ectopic Production of hCG and Free Subunits by Nontrophoblastic Tumors**

The following table shows results obtained in various tumors and healthy and benign disease controls:
Measurement of hCG, α-hCG, and β-hCG serum levels in nontrophoblastic tumors, benign disease, and healthy controls

Tumor type	No. of samples	hCG (ng/mL)	α-hCG (ng/mL)	β-hCG (ng/mL)
Cervix	20	0	1 (1.6)[a]	1 (0.65)
Corpus uterus	20	0	0	0
Gastric	20	0	0	1 (1.5)
Pancreatic	20	0	1 (16.0)	2 (0.8, 3.1)
Colon	20	0	0	0
Lung	20	0	1 (90.0)	1 (0.7)
Ovarian	20	0	1 (1.8)	0
Prostate	20	0	1 (1.6)	0
Other digestive tract tumor	18	0	0	0
Total [%]	178	0	5 [3]	5 [3]
Benign disease controls	61	0	1 (1.6)	0
Healthy controls	50	0	0	0
Total [%]	111	0	1 [1]	0

[a] The number in parentheses represents the measured value in ng/mL.
The cut-off values for positive results are 1.5 ng/mL for hCG and α-hCG and 0.4 ng/mL for β-hCG.

When compared with healthy control values, all nontrophoblastic cancer patients had hCG concentration within the normal range (~ 0.9 ng/mL). Free subunits were elevated in 10 of 178 patients. It is noteworthy that α-hCG levels in two patients (pancreatic and lung tumors) were relatively high (16 and 90 ng/mL, respectively), whereas the maximum concentration of free β-hCG was only 3.1 ng/mL (pancreatic tumor).

13. PERFORMANCE CHARACTERISTICS

- **Assay Dynamic Range**

The range of the assay is between 0.2–200 ng/mL.

- **Specificity of Antibodies (Cross Reactivity)**

The following substances were tested for cross reactivity of the assay:

Hormone tested	Concentration	Produced Color Intensity Equivalent to free β-hCG in Serum
TSH	25 µIU/mL	< 0.3 ng/mL
FSH	100 mIU/mL	< 0.2 ng/mL
Prolactin	100 mIU/mL	< 0.5 ng/mL
LH	200 mIU/mL	< 1 ng/mL

- **Sensitivity**

The analytical sensitivity of the Biovendor ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the *Zero Buffer* and was found to be 0.2 ng/mL.

- **Reproducibility**

Intra-Assay (=10)
The within assay variability is shown below:

Sample	Mean (ng/mL)	CV (%)
1	4.49	6.78
2	27.75	6.60
3	107.6	5.83

Inter-Assay (=9)
The between assay variability (three different days) is shown below:

Sample	Mean (ng/mL)	CV (%)
1	14.94	8.00
2	26.34	8.03
3	100.94	6.73

- **Recovery**

Recovery of the Biovendor ELISA was determined by adding increasing amounts of the analyte to two different patient sera containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples.

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		25.73	97.12	99.88
Average Recovery [%]		101.8	90.5	99.88
Range of Recovery [%]	from	98.4	86.2	93.7
	to	109.4	94.9	99.4

- **Linearity**

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		28.30	79.27	185.14
Average Recovery [%]		102.5	105.7	104.9
Range of Recovery [%]	from	99.3	103.4	92.9
	to	109.3	109.1	113.0

14. LIMITATION

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.

- **Interfering Substances**

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

- **Drug Interferences**

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

- **High-Dose-Hook Effect**

No hook effect was observed in this test up to 19800 ng/mL of free β-hCG.

15. QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
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.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient 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 Biovendor directly.

16. LEGAL ASPECTS

- **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 Biovendor.

- **Therapeutic Consequences**

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

- **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 to point 11.2. 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.

17. REFERENCES

›› References to CG:

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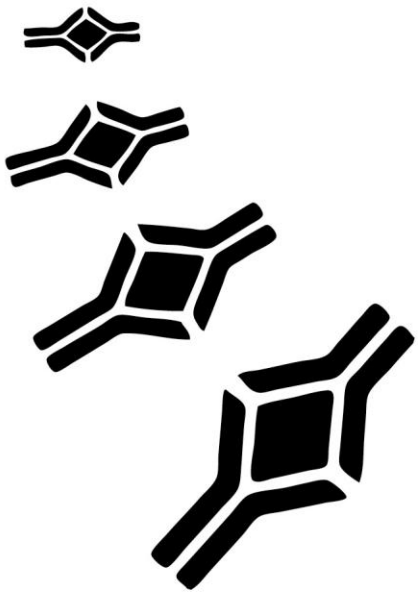
›› For more references on this product see our WebPages at www.biovendor.com



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