AB Diagnostic Systems

REF **DK.043.01.9** Σ **96**

CE

IVD **For *In vitro* Diagnostic Use**

INSTRUCTIONS FOR USE
abia hCG
Enzyme immunoassay for the quantitative determination of human chorionic gonadotropin (hCG) concentration in human serum

This Package Insert provides information for Professional Use of the kit.
The kit contains sufficient reagents for 96 assays (breakable wells) including controls; partial use of the kit is possible; can be used for manual protocol.

I. INTENDED USE

The abia hCG is an enzyme immunoassay for the quantitative measurement of intact human chorionic gonadotropin (hCG) in serum.

This kit is for diagnostic use by a trained laboratory professional and will not be sold to the general public. All the reagents are for professional *in vitro* diagnostic use only.

The results of this or any other diagnostic assay should be used and interpreted only in the context of the overall clinical picture.

II. INTRODUCTION

Chorionic gonadotropin (hCG) is a glycoprotein hormone which is normally produced by the placenta during pregnancy. After conception, the hCG concentration increases rapidly to reach a peak near the end of the first trimester. High concentrations are observed throughout pregnancy. After delivery, hCG levels fall rapidly and become undetectable after a few days. Structurally intact hCG molecules are composed of an alpha and a beta subunits with a molecular weight of 38.4 kDa. The alpha subunit is nearly identical to the alpha subunits of other glycoprotein hormones, such as Thyroid Stimulating Hormone (TSH), Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). The differences in the beta subunit of the respective hormones account for their biological specificity and immunochemical distinctiveness. Monoclonal antibodies recognizing unique sites on the beta chain of the hCG molecule are essential for differentiation between hCG and LH, FSH and TSH. HCG assays are used for the early detection of pregnancy.

In addition to the elevated hCG levels during pregnancy, high concentrations of hCG may be associated with neoplasms of trophoblastic and nontrophoblastic origin such as hydatiform mole, chorionepithelioma, embryonal cell carcinoma, and many others. HCG is commonly elevated in different testicular tumors and is thus used as a tumor marker for testicular tumors in combination with AFP. There is a good correlation between changes of hCG levels and response to therapy.

III. PRINCIPLE OF THE TEST

The abia hCG is a one-step immunoassay, based on principle of “sandwich” method. The assay system utilizes a high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the beta subunit of hCG molecule. The test sample is allowed to react simultaneously with the two antibodies, resulting in the hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with Washing Solution to remove unbound labeled antibodies. A solution of TMB-Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The concentration of hCG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT abia hCG

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-hCG-coated microtiter wells	Polystyrene stripped 96-well plate (breakable wells) coated with monoclonal antibodies to hCG. Once opened, microtiter wells should be stored at 2-8 °C until expiration date of the kit.	1 plate
Conjugate	Monoclonal anti-hCG antibodies conjugated to horseradish peroxidase. Transparent or opalescent pink liquid. Preserving agents: 0.10% ProClin 300, 0.004% gentamycin sulfate. Once opened, Conjugate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 12.0 ml
Calibrator 0 Calibrator 1 Calibrator 2 Calibrator 3 Calibrator 4	Five vials containing hCG in protein-based buffer. Calibrators were calibrated using a WHO 4th IS 75/589. The hCG concentration levels in Calibrators are provided on the labels of vials and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent pale yellow liquids. Preserving agents: 0.05% ProClin 300, 0.10% phenol, 0.004% gentamycin sulfate. Once opened, Calibrators should be used within two months. Store at 2-8 °C in tightly sealed vials.	4 vials 0.5 ml. Calibrator 0 – 2.0 ml
Control Serum	Control Serum containing hCG in protein-based buffer. The hCG concentration level in Serum is provided on the vial label and in the Certificate of Analysis on a lot-specific basis. Transparent or slightly opalescent pale yellow liquid. Preserving agents: 0.05% ProClin 300, 0.10% phenol, 0.004% gentamycin sulfate. Once opened, Control Serum should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 0.5 ml
Sample Diluent	Sample Diluent for dilution of high serum samples. Transparent or slightly opalescent light blue liquid with allowable sedimentation completely dissolved when shaking. Preserving agent: 0.10% ProClin 300. Once opened, Sample Diluent should be stored at 2-8 °C during shelf-life of the kit.	2 vials 15.0 ml
Washing Solution (concentrated 25-fold)	Phosphate-saline solution (pH 7.4-7.7). Transparent or slightly opalescent liquid, colorless, or pale yellow, sediment may form that dissolves completely at 35-39 °C and shaking. Once opened, Washing Solution should be stored at 2-8 °C during shelf-life of the kit.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine (0.03%) in citric acid buffer, containing H_2O_2 (0.01%). Transparent colorless liquid. Once opened, TMB-Substrate should be used within two months. Store at 2-8 °C in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2M sulfuric acid solution. Transparent colorless liquid. Once opened, Stopping Reagent should be stored at 2-8 °C during shelf-life of the kit.	1 vial 25.0 ml
Plate for preliminary dilution of sera	Polystyrene plate with transparent wells.	1 plate

Additionally the following may be included in the delivery set:

- a lid for polystyrene 96-well plates or a protective film for EIA plates;
- disposable tips;
- a plastic dish for liquid reagents;
- polyethylene bag with a Zip-Lock.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following requirements:

- The temperature in the lab should be 18-24 °C.
- Inspect the contents of the box: check the vials and labels integrity. In case of label loss or labels/vials damage, vials should be disposed and **kit cannot be used.**
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or TMB-Substrate.
- Use a new distribution tip for each sample.
- Do not reuse protective films for EIA plates.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and other solutions.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure.
- Use distilled or deionized water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.
- Once the assay has been started, all subsequent steps should be performed without interruption.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "*in vitro* diagnostic use".
- Human origin material used in the preparation of Calibrators and Control Serum has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2).
- Certain reagents contain biological material of animal origin.

- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patients samples as if capable of transmitting infectious disease.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.
- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- All materials contacted with specimens or reagents, including liquid and solid wastes, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations.

Warning!

Conjugate, Calibrators 0-4, Control Serum, Sample Diluent contain ProClin 300.

H317: May cause an allergic skin reaction.
P261: Avoid breathing vapours.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

Danger!

Stopping Reagent contains 0.2M sulfuric acid.

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/ physician.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Distilled or deionized water.
- Automatic or semiautomatic, adjustable or preset single-channel and multi-channel pipettes with a changeable volume for a set of liquids.
- Disposable pipette tips.
- Automatic microplate washer.
- Microplate reader equipped with 450 and 405-415 nm filter.
- Open type automated analyzer with 450 and 405-415 nm filter (for automated procedure).
- Laboratory clock.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Blood samples should be collected according to the current practices. Serum only may be used. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Store/transport the samples in accordance with the current regulatory documentation. If samples are to be stored/transported for a longer period of time, they must be frozen at or below -20 °C. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed bacterial growing, hemolysis, hyperlipidemia and which were preserved by sodium azide must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. Ready to use reagents:

- **Anti-hCG-coated microtiter wells.** Each 12-strips plate (breakable wells) is wrapped in a sealed foil-lined bag. Open the bag and remove the plate. Select the number of strips/wells required for the assay. Place the unused strips/wells back into the foil-lined bag; reseal the foil-lined bag in a Zip-Lock plastic bag. Do not remove desiccant.
- **Calibrators 0-4;**
- **Control Serum;**
- **Sample Diluent;**
- **Conjugate;**
- **TMB-Substrate;**
- **Stopping Reagent.**

2. Reagents to prepare:

- **Working Washing Solution**. Thoroughly shake Washing Solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with distilled or deionized water (1:24 ratio) in a separate vial. The prepared Working Washing Solution is stable for 14 days at room temperature or for 28 days at 2-8 °C.

X. SPECIMEN DILUTION

If in an initial assay, a specimen is found to contain more than the highest calibrator, the specimens can be diluted by Sample Diluent in the Plate for preliminary dilution of sera and reassayed as described in TEST PROCEDURE. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:20: 10 µl Serum + 190 µl Sample Diluent (mix thoroughly).

b) dilution 1:400: 10 µl dilution a) 1:20 + 190 µl Sample Diluent (mix thoroughly).

c) dilution 1:2000: 20 µl dilution b) 1:400 + 80 µl Sample Diluent (mix thoroughly).

XI. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature for 30 min.

1. To the wells add 25 µl of Calibrators and Control Serum in duplicate. Leave two wells for OD control of TMB-Substrate (blank).

2. To the rest of the wells, add 25 µl of samples in duplicate. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Add 100 µl of Conjugate to all wells except for the wells for OD control of TMB-Substrate (blank).

4. Swirl the microplate gently for 30 seconds after adding of samples and Conjugate to mix, cover the strips with a lid or a protective film and incubate for 90 minutes at room temperature (here 20-25 °C).

5. Aspirate the contents of the wells into the container with disinfecting solution. Wash the wells 5 times with 300 µl of Working Washing Solution per well and remove Working Washing Solution using a washer into the container with disinfecting solution. Tap the plate firmly against absorbance paper to ensure that it is dry – the residual volume must be lower than 10 µl (the use of a washer is recommended). Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.

6. Pipette 100 µl of TMB-Substrate into each well.

7. Incubate for 20-30 minutes at room temperature in a dark place.

8. Pipette 150 µl of Stopping Reagent into each wells. Gently mix for 5-10 seconds.

9. Read the plate on microplate reader at 450 nm. In case of overflow absorbance values, read at 405-415 nm. Reading must be completed within 20 minutes after addition of the Stopping Reagent.

Scheme of the assay is represented in Annex.

Spectrophotometric verification of reagent pipetting

The presence of Conjugate + sample in the well can be verified by automatic reading at 540 (550) nm. Each well containing sample and Conjugate must have an OD higher than 0.500.

10. Automated analyzers

Validated test protocols and dilution tables of reagent working solutions for different EIA-analyzers can be obtained from the manufacturer upon request (see section XV). For the instrumentation without established validated protocol follow the section “TEST PROCEDURE” and ensure all requirements described in the section “PRECAUTIONS” are fulfilled. All protocols for automated analyzers must be fully validated before use.

When preparing working reagent solutions for automated EIA procedure, it is necessary to consider “dead” volume of vials and containers used for loading working solutions in the EIA analyzer.

XII. CALCULATION OF RESULTS

1. Calculate the mean absorbance value of each calibrator duplicate.
2. Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
3. Calculate the mean absorbance values for each specimen.
4. Read the values of the unknowns directly off the calibration curve, If immunoassay software is being used, a 4-parameter curve is recommended.
5. In case of overflow absorbance values at 450 nm, read the results at 405-415 nm.
6. If a sample reads more than value of Calibrator 4 then dilute it by Sample Diluent as described in SPECIMEN DILUTION. The result obtained should be multiplied by the dilution factor.

Typical tabulated data

Calibrator	OD1	OD2	Mean OD-blank	Value (mIU/ml)
0	0.047	0.045	0	0
1	0.178	0.185	0.136	25
2	0.572	0.589	0.535	100
3	1.313	1.413	1.317	250
4	2.234	2.266	2.204	500
Unknown	0.384	0.372	0.332	63

This data is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Blank OD:** The absorbance value should not be more than 0.1 at 450 nm.
2. The absorbance (OD) of **Calibrator 4** should not be less than 1.3 at 450 nm.
3. Calculated Value of **Control Serum** should be within established range.

XIII. PERFORMANCE CHARACTERISTICS OF abia hCG

1. Assay Dynamic Range

The range of the assay is between 0-500 mIU/ml.

2. Analytical sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 12 replicate analyses) plus 2 SD.

Therefore, the sensitivity of the abia hCG kit does not exceed **1 mIU/ml.**

3. Specificity (cross reactivity)

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

Substance	hCG	FSH	TSH	LH
Cross reactivity	100%	0.1%	0.0000%	0.9%

4. Precision

Intra-assay precision

The within assay variability is shown below:

Sample	**n**	**Mean, mIU/ml**	**SD**	**CV, %**
1	9	78.1	4.794	6.1

Inter-Assay precision

The between assay variability is shown below:

Sample	**n**	**Mean, mIU/ml**	**SD**	**CV, %**
1	4	77.8	4.308	5.5

5. Recovery

Spiked samples were prepared by adding defined amounts of hCG to patient serum sample. The results are tabulated below:

Added conc., mIU/ml	**Measured conc., mIU/ml**	**Expected conc., mIU/ml**	**Recovery, %**
-	78.1	78.1	
100	89.7	89.1	101

6. Linearity

Three samples were diluted with Calibrator 0. Each dilution was in 9 repeats. The results are tabulated below:

Undiluted	**Dilution**	**Measured Conc., mIU/ml**	**Expected Conc., mIU/ml**	**Recovery, %**
500	500:2	238.2	250.0	95
250	250:2	118.2	125.0	95
100	100:2	49.7	50.0	99

7. Expected normal Value

hCG concentration was measured in blood serum collected from 9 to 11 AM from 200 healthy individuals of the age 21 – 40 years. hCG concentration did not exceed 10 mIU/ml. Table demonstrates the expected hCG levels in women at different periods of normal pregnancy and the respective serum dilutions recommended for analysis.

Gestational age, weeks from conception	2.5 percentile, mIU/ml	Mediane, mIU/ml	95 percentile, mIU/ml	Recommended dilution
1-3	50	7740	15000	Undiluted and 1:20
3-4	1200	20100	30000	1:20 and 1:400
4-5	2500	33800	85000	1:400
5-6	8500	67200	155000	1:400
6-7	12000	108100	215000	1:400 and 1:2000
7-8	35000	132900	255000	1:400 and 1:2000
8-9	40000	149200	280000	1:400 and 1:2000
9-10	35000	137800	250000	1:400 and 1:2000
10-11	30000	114600	205000	1:400 and 1:2000
11-12	16000	100600	195000	1:400
13-14	16000	60400	165000	1:400
15-16	9500	35000	100000	1:400
17-21	7000	30100	75000	1:400

8. Accuracy

The abia hCG kit was compared with a Chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 166. The values ranged from 0 to 785 mIU/ml. The least square regression equation and the correlation coefficient were computed for abia hCG in comparison with the reference method. The least square regression analysis was **y=0.91(x) – 5.69** with correlation coefficient 0.98.

XIV. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the direct determination of hCG in human serum. The kit is not calibrated for the determination of hCG in saliva, plasma or other specimens of human or animal origin.
2. Any improper handing of samples or modification of this test might influence the results.
3. Only sample diluent may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
4. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient.
5. Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.
6. No hook effect was observed in this test to 400000 mIU/ml.

XV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging.

Keep in dark dry place at 2-8 °C. Freezing is prohibited.

Transportation should be done at 2-8 °C. Transportation at 9-20 °C is allowed not more than during ten (10) days.

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XVI. REFERENCES

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2. Braunstein G.D., Vaitukaitis J.L. Carbone P.P. and Ross G.T. Ectopic production of human chorionic gonadotropin by neoplasms. Ann Intern. Med. 1973, 78: 39-45.

3. Cacciatore B., Tiitinen A., Stenman U.H., Ylostalo P. Related Articles, Normal early pregnancy: serum hCG levels and vaginal ultrasonography findings. Br J Obstet Gynaecol. 1990 Oct.; 97(10):899-903.

XVII. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)	+2°C +8°C	Storage temperature limitation
	Manufacturer		Consult Instruction for use
	Date of manufacture CCYY-MM	IVD	For in vitro diagnostic use
	Expiry date CCYY-MM-DD	Σ	Sufficient for
LOT	Batch code		Symbol “exclamation mark”
REF	Catalog number	**Warning!**	Signal word
	Fragile, handle with care		Symbol “corrosion”
	Keep away from sunlight	**Danger!**	Signal word
	Keep dry		Top

Scheme of the assay

1	**Add**	25 µl of Calibrators, Control Serum in duplicates; 25 µl of samples in duplicates; two wells for OD control of TMB-Substrate (blank)
2	**Add**	100 µl of Conjugate into all wells, except for the wells for OD control of TMB-Substrate (blank)
3	**Mix**	30 seconds
4	**Incubate**	90 min, at 20-25 °C
5	**Wash the plate**	Working Washing Solution, 300 µl, 5 times
6	**Add**	100 µl of TMB-Substrate into all wells
7	**Incubate**	20-30 min, at room temperature in a dark place
8	**Add**	150 µl of Stopping Reagent into all wells
9	**Mix**	5-10 seconds
10	**Read the optical density**	450 nm, in case overflow OD values – 405-415 nm