AccuDiag™ hCG Visual ELISA Test Kit

REF 4102-16

IVD See External Label 2°C 8°C Σ 96 Tests

hCG Visual	
Method	Enzyme Linked Immunosorbent
Principle	Sandwich Complex
Detection Range	0-300 mIU/ml
Sample	50 ul
Specificity	96%
Sensitivity	20 mIU/ml
Incubation Time	15 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

For the qualitative determination of hCG in human serum or urine.

SIGNIFICANCE AND SUMMARY

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected in serum as early as 7 days following conception, doubling every 1.3 to 2 days. At the time of the first missed menstrual period, serum hCG concentration is about 100 mIU/ml, and peak levels of 100,000~200,000 mIU/ml are seen at the end of the first trimester. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth make it an excellent marker for the early detection of pregnancy. Elevated serum hCG levels comparable to those observed in early pregnancy may also be associated with trophoblastic or nontrophoblastic neoplasms such as hydatidiform mole, choriocarinoma; therefore, the possibility of such diseases should be ruled out before a positive hCG result is considered diagnostic for pregnancy.
The hCG Visual Test Kits is a rapid test to detect the presence of hCG in urine or serum specimens in a qualitative format.

ASSAY PRINCIPLE

The hCG Visual Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum or urine) is added to the hCG antibody coated microtiter wells and incubated with the hCG antibody labeled with horseradish peroxidase (conjugate). If hCG is present in the specimen, the hCG molecules will be sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for five minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Urine should be fresh. Filter the urine before testing if it looks turbid. This kit is for use with samples without additives only.

REAGENTS

Materials provided with the test kit

1. Antibody-Coated Microtiter Wells, 96 wells
2. Anti-hCG antibody HRPO Conjugate Reagent, 6 ml
3. hCG Standard 0.0 mIU/ml, 1 ml
4. hCG Standard 20.0 mIU/ml, 1 ml
5. hCG Standard 150.0 mIU/ml, 1 ml
6. hCG Standard 300.0 mIU/ml, 1 ml
7. TMB Substrate, 12ml
8. Stop Solution , 12 ml
9. Wash Buffer Concentrate(50X), 15ml

Materials required but not provided

1. Distilled water
2. Precision pipettes: 0.05 ml and 0.1ml
3. Disposable pipette tips
4. Vortex mixer or equivalent
5. Absorbent paper

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18~22°C) before use.
2. Gentle swirl each bottle liquid reagent. Do not shake or agitate reagent bottle vigorously.
3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated well in the holder. Make data sheet with sample identification.
2. Dispense 50 µl of samples, and one drop of standards (50 µl) into appropriate wells. Thoroughly mix for 5 seconds.
3. Incubate at room temperature for about 5 minutes.
4. Remove the incubation mixture by flicking plate contents into a waste container.
5. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
7. Dispense one drop (50 µl) of Enzyme Conjugate Reagent into each well. Thoroughly mix for 5 seconds.
8. Incubate at room temperature for about 5 minutes.
9. Remove the incubation mixture by flicking plate contents into a waste container.
10. Rinse and flick the microtiter wells 5 times with washing buffer (1X).
11. Dispense two drops of TMB Substrate (100 µl) into each well.
12. Gently mix for 5 seconds.
13. Incubate at room temperature for at least for five minutes.
14. Read results and compare the color of the patient sample wells to that of the standard wells.
15. If a quantitative result is expected, stop the reaction by adding two drops (100µl) of Stop Solution to each well.
16. Gently mix for 30 seconds to make sure that the blue color changes to yellow color completely.
17. Read optical density at 450nm with a microtiter reader within 15 minutes.
18. Compare the readings of samples to that of the standards, and record the result.

Important Note:

1. The wash procedure is critical. Insufficient washing will result in non-proper color development.
2. The standards are calibrated to the WHO 3rd IRP.

RESULTS

The test can be used for the qualitative and quantitative determination of hCG in human serum or urine.

For qualitative determination of hCG
Positive
The positive wells should develop a distinct blue color. Samples that develop color equal to or greater than that of the 20 mIU/ml Control are considered positive.

Negative
Samples producing no color are considered negative. If sample produce more color than zero dose, but less color than 20 mIU/ml, please check the person again to confirm the positive.

For quantitative determination of hCG
Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of hCG in mIU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of typical standard run with optical density reading at 450nm shown in the Y-axis against hCG concentrations shown in the X-axis. This standard curve 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.

hCG (mIU/ml)	Absorbance (450nm)
0	0.005
20	0.215
150	1.096
300	1.765

STORAGE

1. Store the kit at 2 to 8°C upon receipt and when it is not in use.
2. Keep microtiter wells in a sealed bag with desiccants.

LIMITATIONS

There are some limitations of the assay:

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Brand Name	AccuDiag™
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
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