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IVD | See external label | 2°C-8°C | Σ=96 tests | REF Cat # 4102-16

MICROWELL ELISA
HUMAN CHORIONIC GONADOTROPIN
(HCG) VISUAL TEST KIT

hCG Visual

Cat # 4102-16

ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETERMINATION OF HUMAN CHORIONIC GONADOTROPIN (hCG) IN HUMAN SERUM OR URINE.

Test	**HUMAN CHORIONIC GONADOTROPIN**
Method	**Enzyme Linked Immunosorbent**
Principle	**Peroxidase – Conjugated Sandwich ELISA**
Detection Range	**0-300mIU/ml**
Sample	**50 ul**
Specificity	**96%**
Sensitivity	**20 MIU/ml**
Total Time	**~ 10 min**
Shelf Life	**12-14 months**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended Use

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

Introduction

Diagnostic Automation 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.

Principle of the test

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 microtiterwells 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.

Materials Provided with Test Kit

1. Antibody-Coated Microtiter Wells, 96 wells
2. Anti-hCG antibody HRPO Conjugate Reagent, 6 ml
3. hCG Control I 0.0 mIU/ml, 1 ml
4. hCG Control II 20.0 mIU/ml, 1 ml
5. hCG Control III 150.0 mIU/ml, 1 ml
6. hCG Control IV 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

Storage

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

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

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.

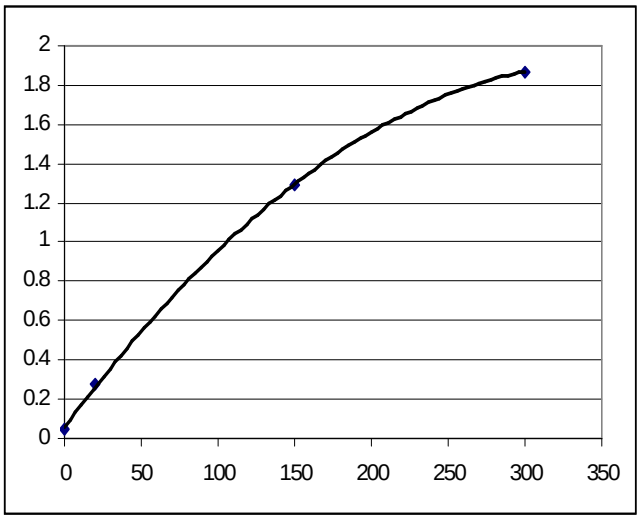
Assay Procedure

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

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	0.045
20	0.273
150	1.293
300	1.870



Interpretation of Results

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.

Important Note

The wash procedure is critical. Insufficient washing will result in non proper color development.

References

1. Stenman U.H., Tanner P., Ranta T., Schroder J. and Seppala M. **Obstet. Gynecol.** 1882; 59:375-377
2. Kosasa T.S. **J. Reprod. Med.** 1981; 26: 201
3. Dipietro D.L. **Laboratory Management** 1981; 19: 1
4. Uotilia M., Ruoslahti E. and Engvall E. **J. Immunol. Methods** 1981; 42: 11-15
5. Masseyeff R. and Maiolini R. **J. Immunol Methods** 1975; 8: 233

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