HUMAN CHORIONIC GONADOTROPIN (HCG) VISUAL PREGNANCY

ENZYME IMMUNOASSAY TEST KIT
(Stat hCG 96 Tests)
Catalog Number: 10011-1

Enzyme immunoassay for the qualitative and quantitative determination of human chorionic gonadotropin (HCG) in human serum or urine.
(For In Vitro Diagnostic Use Only)

Intended Use

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

Introduction

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 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 a incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 5 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

- Antibody-Coated Microtiter Wells, 96 wells .
- Anti-hCG antibody HRPO Conjugate Reagent, 7 mL.
- hCG Standard 0.0 mIU/mL, 1mL.
- hCG Standard 20.0 mIU/mL, 1 mL.
- hCG Standard 50.0 mIU/mL, 1 mL.
- hCG Standard 150.0 mIU/mL, 1 mL.
- hCG Standard 300.0 mIU/mL,1 mL.
- hCG Standard 1000.0 mIU/mL, 1 mL.
- TMB Substrate, 12 mL .
- Stop Solution , 12 mL.
- Wash Buffer(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

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

Reagent Preparation

1.All reagents should be allowed to reach room temperature (18~25°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 Concentrate (50x) into 735ml of 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 50μL of samples, and one drop of standards (50μL) into appropriate wells. Thoroughly mix for 5 seconds.

3.Incubate at room temperature for about 5 min.

4.Remove the incubation mixture by flicking plate contents into a waste container.

5.Rinse and flick the microtiter wells 5 times with wash buffer (1X) .

6.Strike the wells sharply onto absorbent paper to remove residual water droplets.

7.Dispense 1 drop (50μL) of Enzyme Conjugate Reagent into each well. Thoroughly mix for 10 seconds. Incubate at room temperature for about 5 minutes.

8.Remove the incubation mixture by flicking plate contents into a waste container.

9.Rinse and flick the microtiter wells 5 times with wash buffer (1X).

10.Strike the wells sharply onto absorbent paper to remove residual water droplets.

11.Dispense two drops (100μL) of TMB substrate into each well.

12.Gently mix for 5 seconds.

13.Incubate at room temperature in the dark at least for 5 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 one drop (50μL) 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 Notes

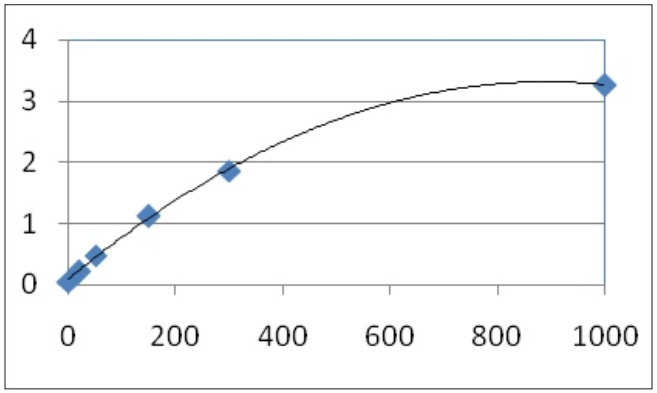
1.The wash procedure is critical. Insufficient washing will result in non-proper color development.

2.The Standards are calibrated to the WHO 3rd IRP.

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	0.054
20	0.222
50	0.482
150	1.188
300	1.812
1000	3.260



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 standard 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 people again to confirm the positive.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

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 to eliminate all the effects of that.

3)The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

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Chemux Bioscience,Inc.

Website: www.chemux.com

385 Oyster Point Blvd Suite5-6.,South San Francisco,CA94080
Tel:+1-650-872-1800