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USA: **RUO**

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**Rapid Visual ELISA Test for Determination of Human Chorionic Gonadotropin (hCG) in Urine or Serum**

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**This kit is intended for Research Use Only.****Not for use in diagnostic procedures.****INTENDED USE**

For determination of Human Chorionic Gonadotropin (hCG) in human urine or serum.

**PRINCIPLE OF THE TEST**

The Visual hCG ELISA Test is a sandwich enzyme immunoassay (8-9) for the determination of human chorionic gonadotropin in urine or serum. The method employs two monoclonal antibodies to selectively identify hCG in urine/serum with a high degree of sensitivity. In less than 10 minutes, elevated levels of hCG as little as 20 mIU/ml can be detected.

The specimen is allowed to react with the antibody enzyme conjugate and the antibodies on the solid phase simultaneously. In the presence of hCG, a specific antibody-hCG-antibody-enzyme complex will form on the surface of microtiter well. After unbound enzyme conjugate is removed by rinsing under a stream of distilled water, the well is incubated with TMB Reagent. The development of blue color in the well indicated the presence of hCG.

Comparing the color intensity of donor samples with that of the provided known reference, the amount of hCG can be visually estimated to be greater or less than 20 mIU/ml.

**REAGENTS*****Materials provided with the kit:***

1. Microtiter Wells: mouse monoclonal anti- $\alpha$ -hCG coated wells, 96 wells.
2. Enzyme Conjugate: containing mouse monoclonal anti- $\beta$ -hCG peroxidase conjugate in protein stabilizer, 7 ml (Red cap).
3. HCG Standard: containing 0 mIU/ml hCG, 1 ml (White cap).
4. HCG Standard: containing 20 mIU/ml hCG, 1 ml (Yellow cap).
5. HCG Standard: containing 150 mIU/ml hCG, 1 ml (Black cap).
6. TMB Reagent (One-Step), 7 ml (Amber cap).
7. Stop Solution: 1N HCl, 7 ml (Natural cap).

***Materials required but not provided:***

- Specimen collection containers
- Timer
- Distilled or deionized water
- Absorbent paper towels

**STORAGE INSTRUCTIONS**

1. Store reagents at refrigerator temperature (2-8°C) when not in use. **Do not freeze.**
2. Bring reagents and specimens to room temperature (18-25°C) before testing.



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USA: **RUO****TEST PROCEDURE**

All reagents and specimens must be brought to room temperature and mixed thoroughly before beginning the test.

**A. Qualitative ELISA Testing**

1. Place Microtiter Wells for your test on the holder.
2. Dispense 1 drop (50 µl) of hCG of donor sample and/or 1 drop (50 µl) of hCG Standards and Negative Reference, if desired, into the appropriately labeled Microtiter Wells. Use a separate disposable pipette for each specimen.
3. Add 1 drop (50 µl) of Enzyme Conjugate into each well. Mix gently for 10 seconds.
4. Incubate at room temperature for 5 minutes.
5. Remove content by flicking the microtiter well holder into sink, followed by rinsing the wells 5 times with ***distilled or deionized water***. Note: Avoid well to well contamination from water over flow during the first rinse. Separating wells on the well holder would help.
6. Add **1 drop** of TMB Reagent into each well. Mix gently for 10 seconds.
7. Incubate at room temperature for 5 minutes.
8. Compare the color developed in specimen wells to that of the positive reference well (20 mIU/ml).

**B. Quantitative Reader Procedure**

In order to run a standard curve, test hCG standards included in the kit by the same method as the test specimens.

Following step 7 in Procedure A, if a microtiter reader is available for quantitative reading at 450 nm, proceed immediately after five minutes incubation:

1. Rapidly add **1 drop** (50 µl) of Stop Solution (1N HCl) into each well including your test specimen, all hCG Standards and Negative Reference.
2. Read absorbance at 450 nm with a microtiter well reader **within 15 minutes**.
3. Calculate the hCG concentration from the standard curve.

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