Foresight®

Rapid hCG EIA Test Kit
Package Insert

REF	I231-4011	English

An enzyme immunoassay (EIA) for the qualitative and semi-quantitative detection of hCG in human serum or urine.
For professional in vitro diagnostic use only.

INTENDED USE

The Rapid hCG EIA Test Kit is a one step enzyme immunoassay for the qualitative and semi-quantitative detection of human chorionic gonadotropin (hCG) concentration in human serum or urine.

SUMMARY

During normal pregnancy, human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine. hCG is made by cells that form the placenta. It is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the diagnosis of early pregnancy disorders. hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/mL by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/mL (approximately 28 days after conception).[1] A peak of 50,000 or even 100,000 mIU/mL is attained by the third month, then a gradual decline is observed.[2,3] In this method, hCG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of hCG) are added and the reactants mixed. Reaction between the various hCG antibodies and native hCG forms a sandwich complex that binds with the streptavidin coated to the well. After the completion of the required incubation period, the enzyme-chorionic gonadotropin antibody bound conjugate is separated from the unbound enzyme-chorionic gonadotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known chorionic gonadotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with chorionic gonadotropin (hCG) concentration.
The Rapid hCG EIA Test Kit is an immunoassay for the qualitative and semi-quantitative detection of the presence of hCG in human serum or urine specimen. The test utilizes monoclonal antibodies to selectively detect hCG in serum or urine.

PRINCIPLE

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of anti-hCG antibody (MoAb) coated on the well and exogenously added anti-hCG. Upon mixing anti-hCG antibody (MoAb), the enzyme-labeled anti-hCG and the sample containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$EnzAb + AghCG + Ab \underset{K\text{-}a}{\overset{Ka}{===}} EnzAb\text{-}AghCG\text{-}Ab$$

Ab= anti-hCG(MoAb) (Excess Quantity)
AghCG= Native Antigen (Variable Quantity)
EnzAb= Enzyme labeled Goat α hCG (Excess Quantity)
EnzAb-AghCG-Ab =Ag-Antibodies Sandwich complex
Ka = Rate Constant of Association
K-a= Rate Constant of Dissociation

PRECAUTIONS

- This kit is NOT intended to be used for the risk evaluation of trisomy 21.
- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipette tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- The calibrators of this kit were human urine based. No known test method can offer complete assurance that products derived from human tissue or excretion will not transmit infectious agents. Therefore, all human derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The Rapid hCG EIA Test Kit can be performed using only human serum or urine.
- Separate serum from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and urine specimens may be stored at 2-8°C for up to 2 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity	
			96 wells/kit	480 wells/kit
	Rapid hCG Microwell plate	Microwell plate coated with anti-hCG	1 plate (96 wells/plate)	5 plates (96 wells/plate)
1	Rapid hCG Conjugate	Anti-hCG bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL
2	Concentrated Wash Buffer(25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 40 mL	5 x 40 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	Rapid hCG Calibrator 1	Buffer containing conjugate stabilizer Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
7	Rapid hCG Calibrator 2	Diluted human Chorionic Gonadotropin containing 25 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
8	Rapid hCG Calibrator 3	Diluted human Chorionic Gonadotropin containing 50 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
9	Rapid hCG Calibrator 4	Diluted human Chorionic Gonadotropin containing 100 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
10	Rapid hCG Calibrator 5	Diluted human Chorionic Gonadotropin containing 250 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5 mL	5 x 0.5 mL
	Plate Sealers		2	10
	Package Insert		1	1

Note: The calibrators were calibrated using a reference preparation, which was assayed against the World Health Organization International Standard for human chorionic gonadotropin (4th International Standard preparation).

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 20°C to 30°C.
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Automated processor (optional)
- Calibrated micropipettes with disposable tips capable of dispensing 20, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Timer

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (20-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the Calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle in a graduated cylinder and fill it with freshly distilled or deionized water to 1000 mL for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 20-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 20 µL of Calibrator 1 in wells B1 and C1. • Add 20 µL of Calibrator 2 in wells D1 and E1. • Add 20 µL of Calibrator 3 in wells F1 and G1. • Add 20 µL of Calibrator 4 in wells H1 and A2. • Add 20 µL of Calibrator 5 in wells B2 and C2. The colors of Calibrators 1-5 gradually change from colorless to blue.	• B1 and C1: Add 20 µL Calibrator 1 • D1 and E1: Add 20 µL Calibrator 2 • F1 and G1: Add 20 µL Calibrator 3 • H1 and A2: Add 20 µL Calibrator 4 • B2 and C2: Add 20 µL Calibrator 5
2	• Add 20µL of specimen to assigned wells start at D2 (or D2 AND E2). Duplicate-well addition is recommended.	• Starting D2: Add 20 µL specimen
3	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Conjugate to each well except for the Blank well
4	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds.	• Mix gently • Cover the microwell plate with the

	• Cover the microwell plate with the Plate Sealer and incubate at room temperature (20-30°C) in a room, a water bath, or an incubator for 10 minutes.	Plate Sealer and incubate at room temperature (20-30°C) for 10 minutes
5	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
6	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) A blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
7	• Mix gently then cover microwell plate with Plate Sealer and incubate at room temperature (20-30°C) in a room, a water bath, or an incubator for 5 minutes. • Remove the Plate Sealer. **For Qualitative detection**: It's recommended to interpret the results directly without adding the stop solution. **For Semi-Quantitative detection**: Continue with following Step 8 and 9.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature (20-30°C) for 5 minutes • Remove the Plate Sealer **Qualitative detection**: Interpret the results directly. **Semi-Quantitative detection**: Continue with following Step 8 and 9.
8	• Add 50 µL of Stop Solution to each well. (Clear Reagent) A yellow color should develop in wells containing Positive specimens.	• Add 50 µL of Stop Solution to each well
9	• Read at 450/630-700 nm within 15 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 15 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Calibrators 1-5 by referring to the table below.

Example of Calibrator 2 Calculation

Item	Absorbance
Calibrator 2: Well D1	0.404
Calibrator 2: Well E1	0.394
Total Absorbance of Calibrator 2	0.404 + 0.394 = 0.798
Mean Absorbance of Calibrator 2	0.798/2 = 0.399
Blank Absorbance: Well A1	0.028
Mean Absorbance of Calibrator 2 – Blank Absorbance	0.399 – 0.028 = 0.371

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Calibrator 1	Mean Absorbance after subtraction of Blank Absorbance should be < 0.105
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > 0.105
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > 0.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Semi- quantitative

Draw the calibration curve and obtain semi-quantitative specimen results.

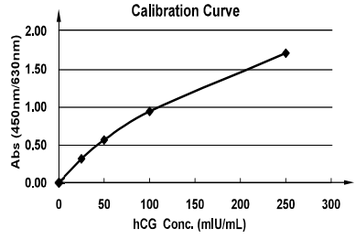
1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, and then plot them on the Y-axis against their concentration in mIU/mL on the X-axis on a linear graph paper and draw the calibration curve. Draw the best fitted line through data points to obtain a standard curve. Refer to an example of the calibration curve at right.

NOTE: Do not use the calibration curve above to make any calculation. A calibration curve must be performed for each run.

2. Obtain quantitative specimen results from their absorbance after subtraction of Blank Absorbance by using the calibration curve.

Qualitative

1. It is recommended that for visual qualitative detection to interpret the results directly without adding the stop solution. Blue color is much easier for interpretation than the yellow color which will be produced after the addition of Stop Solution. In this case, make sure to interpret the results immediately. Delay in the interpretation may cause false positive.
2. Compare the color intensity of the specimen wells to the color intensity of the wells for Calibrator 2 (25 mIU/mL).
3. If the color intensity of the specimen wells is lower than the color intensity of the wells for Calibrator 2, meaning the hCG concentration of the specimen is lower than 25 mIU/mL, then the specimen should be considered as Negative.
4. If the color intensity of the specimen wells is higher than the color intensity of the wells for Calibrator 2, meaning the hCG concentration of the specimen is higher than 25 mIU/mL, then the specimen should be considered as Positive.



LIMITATIONS

1. The Rapid hCG EIA Test Kit is used for the detection of hCG in human serum or urine.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that non-repeatable reactive results may occur due to inadequate washing. The results may be affected due to procedural or instrument error.
4. Erroneous result may be due to fibrin particles and microbial contamination.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the Rapid hCG EIA Test Kit is 0.68 mIU/mL.

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of three specimens with hCG concentration at 50 mIU/mL, 100 mIU/mL, and 250 mIU/mL respectively.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens with hCG concentration at 50 mIU/mL, 100 mIU/mL, and 250 mIU/mL respectively. Three different lots of the Rapid hCG EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance	Standard Deviation	Coefficient of Variation (%)
1	0.389	0.039	10.03	0.534	0.07	13.11
2	0.856	0.074	8.64	0.987	0.123	12.46
3	1.54	0.108	7.01	1.756	0.176	10.02

Accuracy

The Rapid hCG EIA Test Kit has been compared to a leading commercial Rapid hCG EIA test using clinical specimens. A total of 66 serum specimens and 70 urine specimens ranging from 0~250mIU/mL were run and analyzed using least square regression analysis. The results show that the Rapid hCG EIA Test Kit has good correlation compared to the reference method.

Specimens	No.	Range (mIU/mL)	Slope	Correlation Coefficient
Serum	66	0~250	0.95	0.99
Urine	70	0~250	0.53	0.94

Dose Hook Effect

No dose hook effect is observed up to 25000 mIU/mL of hCG.

Interferences and Cross-Reactivity

The specificity of the Rapid hCG EIA Test Kit was determined by testing sera containing the compounds listed below. These compounds showed less than 20% interference in the Rapid hCG EIA Test Kit at the levels indicated.

Substance	Concentration	Substance	Concentration
Hemoglobin	12 mg/mL	Ascorbic Acid	100 µg/mL
Albumin	60 µg/mL	triglycerides	100 µg/mL
cholesterol	100 µg/mL		

The following substances and concentrations have also been tested using Rapid hCG EIA Test Kit and no interference was observed.

Substance	Concentration	Substance	Concentration
Follitropin (hFSH)	1000 mIU/mL	TSH	1000 µIU/mL
LH	300 mIU/mL		

BIBLIOGRAPHY

1. Kosasa TS., "Measurement of Human Chorionic Gonadotropin", *Journal of Reproductive Medicine,* 26, 201-6 (1981).
2. Danzer H., et al., "Maternal Serum Human Chorionic Gonadotropic Concentrations and Fetal Sex Predictions*", Fertility and Sterility,* 34, 336-40 (1980).
3. Braunstein G.D., et al., "Serum Human Chorionic Gonadotropin Levels through Normal Pregnancy", *American Journal of Obstetrics and Gynecology* 126, pg. 678-81 (1976).

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	EC REP	Authorized Representative
2°C 8°C	Store between 2-8°C	LOT	Lot Number	REF	Catalog #
Rapid hCG	Rapid hCG	Substrate A	Substrate A	Substrate B	Substrate B
Wash Buffer 25x	Wash Buffer (25x)	Conjugate	Conjugate	Calibrator 1	Calibrator 1
Calibrator 2	Calibrator 2	Calibrator 3	Calibrator 3	Calibrator 4	Calibrator 4
Calibrator 5	Calibrator 5	Stop Solution	Stop Solution	Package Insert	Package Insert
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		

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