Foresight®

hCG EIA Test Kit
Package Insert

REF	I231-4051	English

An enzyme immunoassay (EIA) for the quantitative detection of hCG (human chorionic gonadotropin) in human serum.

For professional in vitro diagnostic use only.

INTENDED USE

The hCG EIA Test Kit is an enzyme immunoassay for in vitro quantitative determination of hCG level in human serum. It is intended as an aid in the early detection of pregnancy.

SUMMARY

Human chorionic gonadotropin (hCG) is a sialoglycoprotein with a molecular weight of approximately 46,000 Daltons [1]. The hCG molecule consists of two combined, dissimilar subunits designated alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 Daltons, confers biological and immunological specificity to the entire hCG molecular by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 Daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones; LH, FSH and TSH.

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. According to the literature, 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) [2]. A peak of 50,000 or even 100,000 mIU/mL is attained by the third month, then a gradual decline is observed [3].

With the availability of sensitive quantitative assays for the measurement of serum hCG, it has been shown that hCG levels can be useful in prediction of spontaneous abortions, aiding in the detection of ectopic pregnancy and multiple gestation.

PRINCIPLE

The hCG EIA Test Kit is a solid phase enzyme immunoassay based on a sandwich principle for the quantitative detection of hCG in human serum. The microwell plate is coated with monoclonal antibodies specific to hCG. During testing, the specimen and the enzyme-conjugated hCG antibodies are added to the antibody coated microwell plate and then incubated. If the specimen contains hCG, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-hCG-conjugate complexes. If the specimen does not contain hCG, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and Substrate B are added and then incubated to produce a blue color, indicating the amount of hCG present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which corresponds to the amount of hCG present in the specimen, is measured with a microplate reader at 450/630-700 nm or 450 nm. The absorbance of the specimen is then compared to a calibration curve to obtain the amount of hCG present in the specimen.

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.
- Add all the calibrators and specimens into the wells within 15 minutes to minimize the change in absorbance which may affect the 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 pipet 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

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood 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 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 hCG EIA Test Kit can be performed using only human serum collected from venipuncture whole blood.
- 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 specimens may be stored at 2-8°C for up to 7 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
	hCG Microwell Plate	Microwell plate coated with monoclonal Anti-hCG	1plate (96wells/plate)	5 plates (96 wells /plate)
1	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	hCG Calibrator 1	Buffer containing conjugate stabilizer Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL
7	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	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	hCG Calibrator 4	Diluted human Chorionic Gonadotropin containing 100 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5mL	5 x 0.5 mL
10	hCG Calibrator 5	Diluted human Chorionic Gonadotropin containing 250 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5mL	5 x 0.5 mL
11	hCG Calibrator 6	Diluted human Chorionic Gonadotropin containing 500 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5mL	5 x 0.5 mL
12	hCG Calibrator 7	Diluted human Chorionic Gonadotropin containing 1000 mIU/mL Preservative: 0.1% ProClin™ 300	1 x 0.5mL	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 25, 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 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 containing the concentrated wash buffer 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 15-30°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 . • Add 20 µL of Calibrator 6 in wells D2 and E2. • Add 20 µL of Calibrator 7 in wells F2 and G2. The colors of Calibrator 1-7 gradually change from yellow to blue.	• B1and C1: Add 20 µL Calibrator 1 • D1and E1: Add 20 µL Calibrator 2 • F1and G1: Add 20 µL Calibrator 3 • H1and A2: Add 20 µL Calibrator 4 • B2and C2: Add 20 µL Calibrator 5 • D2and E2: Add 20 µL Calibrator 6 • F2and G2: Add 20 µL Calibrator 7
2	• Add 20 µL of specimen to assigned wells starting at H2.	• Starting H2: 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
4	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer, and incubate at room temperature (20-30°C), in a water bath or in an incubator at 20-30°C for 60 minutes ± 5 minute.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at room temperature (20-30°C) for 60 min
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) Then a light blue to blue color should develop in wells corresponding to the amount of hCG present in the specimen.	• 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 water bath or in an incubator at 20-30°C for 15 minutes ± 2 minutes.	• Mix then cover microwell plate with Plate Sealer and incubate at room temperature (20-30°C) for 15 min
8	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow should develop in wells containing positive specimens.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
9	• Read at 450/630-700 nm within 30 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 30 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.

QUALITY CONTROL

Control standards are not supplied with this kit; however, it is recommended that normal, low and high controls be tested with each run as a good laboratory practice to monitor assay performance. Each laboratory should establish its own criteria for establishing mean values and acceptable ranges to determine reliability of the results.

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 < 0.050
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 1 and < 0.300
Calibrator 3	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 2 and < Calibrator 4
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 3 and < Calibrator 5
Calibrator 5	Mean Absorbance after subtraction of Blank Absorbance should be > 0.600
Calibrator 6	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 5 and < Calibrator 7
Calibrator 7	Mean Absorbance after subtraction of Blank Absorbance should be > 1.200

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

CALCULATION OF RESULTS

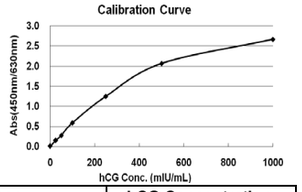
Draw the calibration curve and obtain quantitative specimen results.

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

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

2. Obtain quantitative specimen results of concentrations expressed in mIU/mL from their absorbance by using the calibration curve.

NOTE: Specimens that have absorbance above Calibrator 7 should be pre-diluted using Calibrator 1 and retested. The concentration must be multiplied by the dilution factor. Automated reading and calculation may also be performed using linear regression function on suitable computer programs.



Example of Specimen & Calibrators Result Calculation

Item	Well	Absorbance	Mean (Absorbance-Blank)	hCG Concentration (mIU/mL)
Blank Well	A1	0.004	/	/
Calibrator 1	B1	0.012	0.008	0
	C1	0.011		
Calibrator 2	D1	0.154	0.151	25
	E1	0.155		
Calibrator 3	F1	0.274	0.277	50
	G1	0.287		
Calibrator 4	H1	0.599	0.589	100
	A2	0.586		
Calibrator 5	B2	1.230	1.245	250
	C2	1.267		
Calibrator 6	D2	2.071	2.063	500
	E2	2.062		
Calibrator 7	F2	2.650	2.666	1000
	G2	2.689		
Specimen	H2	1.071	1.067	205.278

LIMITATIONS

1. The hCG EIA Test Kit is used for the detection of hCG in human serum. Diagnosis should not be established based on a single test result. Further testing should be performed in assessing clinical status. Specimens containing precipitate may give inconsistent test results.
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 the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.
4. Unusually high titers of heterophilic antibodies or rheumatoid factor (RF) may affect results. Even if test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.

EXPECTED RANGES OF VALUE

A study of non-pregnant females and adult males was conducted to determine expected values for HCG EIA test kit. The Mean (X) value, Standard Deviation (SD) and Expected Ranges (±2SD) are presented in below.

Expected Values for the hCG EIA Test Kit (mIU/mL-5th IS 07/364)

Number	80
Mean (X) Value	1.72
Standard Deviation (SD)	0.5
Expected Ranges (±2SD)	0.72-2.72

Each laboratory must establish its own normal ranges based on patient population. The results above maybe used as initial guideline range.

hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of hCG in the serum of pregnant women increases to 5-50 mIU/mL one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 10,000-200,000 mIU/mL at the end of the first trimester [4].

Although normal pregnancy is usually the cause of increased hCG levels in urine and serum, elevated hCG concentrations have also been reported in patients diagnosed with molar pregnancy, choriocarcinoma, and non-trophoblastic neoplasms [5,6].

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the hCG EIA Test Kit is less than 1.0 mIU/mL.

Reproducibility

Intra-Assay: Intra-Assay precision has been determined by using 10 replicates per run, 2 runs at the morning and afternoon in the same day, total 3 days by different operators.

Inter-Assay: Inter-Assay precision has been determined by using 10 replicates per run, 2 runs at the morning and afternoon in the same day, total 3 days by different operators for three different lots of the hCG EIA Test Kit.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance (mIU/mL)	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance (mIU/mL)	Standard Deviation	Coefficient of Variation (%)
1	23.40	1.82	7.78%	24.26	3.01	12.41%
2	72.24	7.28	10.08%	78.57	8.43	10.73%
3	755.23	52.34	6.93%	783.62	65.28	8.33%

Accuracy

The hCG EIA Test Kit has been compared to a leading commercial hCG EIA test using clinical specimens. A total of 240 clinical specimens ranging from 0~1000 mIU/mL were run and analyzed using least square regression analysis. The results show that the hCG EIA Test Kit has good correlation compared to the reference method.

No. Specimens	Range (mIU/mL)	Slope	Correlation Coefficient
240	0~1000	1.09	0.96

Dose Hook Effect

No dose hook effect is observed up to 100,000 mIU/mL of hCG.

Interferences and Cross-Reactivity

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

Substance	Concentration	Substance	Concentration
Hemoglobin	12 mg/mL	Ascorbic Acid	100 µg/mL
Albumin	6 mg/mL	triglycerides	100 µg/mL
cholesterol	100 µg/mL		

The following substances and concentrations have also been tested using hCG EIA Test Kit and no cross-reactivity was observed.

Substance	Concentration	Substance	Concentration
FSH	1000 mIU/mL	TSH	1000 µIU/mL
LH	1000 mIU/mL		

BIBLIOGRAPHY

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6. Seppala M., et al., “Choriocarcinoma: expression of tumor- and trophoblast-associated antigens in patients with low chorionic gonadotropin excretion”,Cancer 1976:38:2065-2070.

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 #
hCG	hCG	Conjugate	Conjugate	Substrate A	Substrate A
Substrate B	Substrate B	Stop Solution	Stop Solution	Specimen Diluent	Specimen Diluent
Wash Buffer 25x	Wash Buffer (25x)	Calibrator 1	Calibrator 1	Calibrator 2	Calibrator 2
Calibrator 3	Calibrator 3	Calibrator 4	Calibrator 4	Calibrator 5	Calibrator 5
Calibrator 6	Calibrator 6	Calibrator 7	Calibrator 7	Package Insert	Package Insert
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		

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