Foresight®

HCV Antibody Test Kit
(Microplate Chemiluminescence Immunoassay)
Package Insert

REF I531-1031 ***EN*** English

A microplate chemiluminescence immunoassay (CLIA) for the qualitative detection of total antibodies (IgG, IgM and IgA) to Hepatitis C Virus (HCV) in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The HCV Antibody Test Kit (Microplate Chemiluminescence Immunoassay) is a two-step chemiluminescence immunoassay for the *in vitro* qualitative detection of total antibodies (IgG, IgM and IgA) to *Hepatitis C Virus (HCV)* in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible HCV infection.

SUMMARY

Hepatitis C Virus is a small, enveloped, positive-sense, single-stranded RNA virus. HCV is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. HCV infection causes a wide variety of chronic liver disease, cirrhosis and liver cancer. The main route of transmission of the virus is via transfusion of blood and blood products, organ transplantation, and sharing contaminated needles and syringes. Antibodies to HCV is found in over 80% of patients with well-documented non-A, non-B hepatitis. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens.[1,2] Compared to the first generation HCV immunoassay tests using single recombinant antigen, new serologic tests incorporate recombinant protein and/or synthetic peptide antigens to avoid nonspecific cross-reactivity and to increase the sensitivity.[3, 4]
The HCV Test Kit (Microplate Chemiluminescence Method) is a third generation immunoassay for the qualitative detection of the presence of IgG antibodies to HCV in serum or plasma specimen. The test utilizes recombinant HCV antigens encoded by the genes for both structural (nucleocapsid) and non-structural proteins to selectively detect antibodies to HCV in serum or plasma.

PRINCIPLE

The HCV Antibody Test Kit (Microplate Chemiluminescence Immunoassay) is a solid phase qualitative chemiluminescence immunoassay based on a sandwich principle for the detection of total antibodies (IgG, IgM and IgA) to HCV in human serum or plasma. HCV recombinant antigens is bound to the microwell. In the two-step test, the specimen and the enzyme-conjugated HCV antigens are successively added into the microwell and incubated. If the specimen contains antibodies to HCV, it will bind to the antigens coated on the microwell plate and be further combined with the enzyme-conjugate antigens to form immobilized antigen-antibody-antigen-enzyme complexes. If the specimen does not contain antibodies to HCV, the complexes will not be formed. After each incubation, the microwell plate is washed to remove unbound materials. Substrate A and Substrate B, containing chemiluminescence and peroxide respectively are blended completely and then also added into the reaction system. Light signal will be generated and further measured by a photomultiplier (PMT) as relative light units (RLU). Signal intensity is proportional to the concentration of HCV Antibody present in the specimen.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use it 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 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 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

- Human specimens should be considered potentially hazardous. It is recommended that the 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, Specimen Diluent and Controls. 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 exposing Substrate A and Substrate B to direct light, metal or oxidants.
- Non-disposable apparatus should be sterilized after use. The preferred Immunoassay 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 1 month 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 1 month 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.

SPECIMEN COLLECTION AND PREPARATION

- The HCV Antibody Test Kit (Microplate Chemiluminescence Immunoassay) can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and Sodium Citrate collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide (HRP) and may lead to erroneous results.
- Separate serum or plasma 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 plasma 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	96 wells/kit
	HCV Microwell Plate	Microwell plate coated with HCV antigens	1 plate (96 wells/plate)
1	HCV Conjugate	Recombinant HCV antigens bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL
2	Concentrated Wash Buffer (20x)	PBS buffer containing Tween 20; Preservative: 2% ProClin™ 300	1 x 40 mL
2A	Specimen Diluent	Bovine serum (BS); Preservative: 0.1% ProClin™ 300	1 x 6 mL
3	Substrate A	CB buffer containing luminol sodium salt	1 x6 mL
4	Substrate B	PB Buffer containing hydrogen peroxide	1 x 6 mL
5	HCV Negative Control	Bovine serum (BS); Preservative: 0.1% ProClin™ 300	1 x 1 mL
6	HCV Positive Control	BS containing antibodies to HCV and negative for HCV, HBsAg, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300	1 x 1 mL
	Plate Sealers		3
	Package Insert		1

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 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Semi-auto CLIA analyzer, e.g. TZD-CL-200S
- Automated CLIA processor, e.g. Smart 3000

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. From well A1, arrange the controls 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 1:20. 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 800 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 1:20 • Remove and store unused strips at 2-8°C
1	• Add 50 µL of specimen diluent to each well • Add 50 µL of Negative Control in wells A1 and B1. (Blue Reagent) • Add 50 µL of Positive Control in wells C1 and D1. (Red Reagent) • Add 50 µL of specimen to assigned wells starting at E1.	• Add 50 µL of specimen diluent to each well • A1 and B1: Add 50 µLNegative Control • C1 and D1: Add 50 µL Positive Control • Starting E1: Add 50 µL specimen
2	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
3	• Remove the Plate Sealer. • Add 100 µL of Conjugate to each well. (Red Reagent).	• Remove the Plate Sealer • 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 in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
5	• Wash each well 5 times by filling each well with 350 µL of Working Wash Buffer, 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.	• Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
6	• Mix Substrate A and Substrate B in equal volume (Clear Reagent) • Add 100µL of Substrate to each well	• Mix Substrate A and Substrate B • Add 100 µL of Substrate to each well
7	• Mix gently then incubate in the dark at room temperature for 5 minutes.	• Mix then incubate in the dark for 5 min.
9	• Read RLU per well by a chemiluminescence immunoassay analyzer within 30 minutes.	• Read RLU by a chemiluminescence immunoassay analyzer within 30min

AUTOMATED PROCESSING

Automatic CLIA 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 CLIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Cut-Off Value by referring to the table below.
   Cut-off Value (CO) = PC Value *0.01

Example of Cut-Off Value Calculation

Item	RLU
Negative Control: Well A1	1125
Negative Control: Well B1	1135
Positive Control: Well C1	1828200
Positive Control: Well D1	1737875
NC: (Well A1 RLU + Well B1 RLU)/2	1130
PC: (Well C1 RLU + Well D1 RLU)/2	1783038
Cut-Off Value: PC*0.01	17830

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

Item	Validation Requirements
PC/NC	≥100

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

3. Calculate the S/CO Value using the following formula if the test results are valid.

   S/CO = Sample Value/Cut-off Value

Example of Sample Result

Item	Result
Sample 1: Well E1	RLU: 2309125
Cut-off Value	17830
Sample 1 S/CO: Well E1 RLU / Cut-off Value	129.51

INTERPRETATION OF RESULTS

Specimens with S/CO values < 1.00 are considered non-reactive (NR).
Specimens with S/CO values ≥ 1.00 are considered reactive (R).
Specimens with S/CO of 0.9 to 1.1 are handled as gray zone interpretations, further clarification may be obtained by testing another specimen taken three to six weeks later.

LIMITATIONS

1. The HCV Antibody Test Kit (Microplate Chemiluminescence Immunoassay) is used for the detection of *T. Pallidum* antibodies in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. 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 non-repeatable reactive reaction may occur due to inadequate washing. The results may be affected due to procedural or instrument error.
4. The Positive Controls in the test kit are not to be used to quantify assay sensitivity. The Positive Controls are used to verify that the test kit components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HCV Antibody Test Kit has correctly identified specimens of a seroconversion panel and has been compared with a leading commercial HCV Antibody EIA Test Kit using clinical specimens. The results show that the clinical sensitivity of the HCV Antibody CLIA Test Kit is > 99.9%, and the clinical specificity is 99.9%.

HCV Total Antibody CLIA vs. HCV Total Antibody EIA

Method		HCV Total Antibody EIA		Total Results
HCV Total Antibody CLIA	Results	Positive	Negative	
	Positive	58	0	58
	Negative	0	34	34
Total Results		58	34	92

Clinical Sensitivity: 100% (93.84% -100.0%)*
Clinical Specificity: 100% (89.72%-100%)*
Overall Agreement: 100% (96.07%-100%)*
*95% Confidence Interval

The table below shows the results of BBI panel performance with the HCV Antibody CLIA Test Kit.

BBI data sheet				
Panel ID	Days Since 1st Bleed	Abbott ARCHITECT HCV Ab(S/CO)	Abbott PRISM HCV Ab(S/CO)	Acon CLIA HCV Ab(S/CO)
PHV925-01	0	0.02	0.08	0.05
PHV925-02	2	0.02	0.08	0.06
PHV925-03	8	0.03	0.14	0.25
PHV925-04	10	0.03	0.23	1.79
PHV925-05	27	6.85	5.79	142.3

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of a low positive specimen.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same specimen: a low positive. Three different lots of the HCV Total Antibody CLIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean RLU/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean RLU /Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	13.54	1.37	10.1	11.92	1.72	14.5

BIBLIOGRAPHY

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4. Wasserheit JN. *Epidemiological Synergy: Interrelationships Between Human Immunodeficiency Virus Infection and Other Sexually Transmitted Diseases*, Sexually Transmitted Diseases 1992; 19:61-77.
5. Johnson PC. Testing for HCV, Dermatologic Clinic 1994; 12 Jan: 9-17.

Index of Symbols

	Consult instructions for use	Σ	Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	REF	Catalog #
2°C 8°C	Store between 2-8°C	LOT	Lot Number	Substrat B	Substrate B
HCV	HCV	Substrate A	Substrate A	Control +	Positive Control
Wash Buffer 20x	Wash Buffer (20x)	Conjugate	Conjugate	Package Insert	Package Insert
Control −	Negative Control	Specimen Diluent	Specimen Diluent		
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		

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