

HEPATITIS C VIRUS

UBI® HCV EIA
DIRECTION INSERT

FOR *IN VITRO* DIAGNOSTIC USE ONLY

(P/N 100181TW)

CONTENTS

[TRADE NAME & INTENDED USE](#)
[SUMMARY AND EXPLANATION OF THE TEST](#)
[CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE](#)
[REAGENT COMPONENTS AND THEIR STORAGE CONDITIONS](#)
[MATERIALS REQUIRED - NOT SUPPLIED](#)
[WARNINGS AND PRECAUTIONS](#)
[WASTE DISPOSAL](#)
[INTERFERING SUBSTANCES](#)
[SPECIMEN COLLECTION AND PREPARATION](#)
[PREPARATION OF REAGENTS](#)
[STORAGE INSTRUCTIONS](#)
[INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS](#)
[INSTRUMENTATION](#)
[ASSAY PROCEDURE](#)
[RESULTS](#)
[INTERPRETATION OF RESULTS](#)
[LIMITATIONS OF THE PROCEDURE](#)

U.S. Patent No. 5,106,726

U.S. Patent No. 5,436,126

U.S. Patent No. 5,747,239

U.S. Patent No. 5,582,968

Other Patents Worldwide

TRADE NAME & INTENDED USE

UBI[®] HCV EIA is a qualitative enzyme immunoassay for the *in vitro* detection of antibodies to Hepatitis C Virus (HCV) in human serum or plasma. It is intended as a screen for donated blood to prevent transmission of HCV to recipients and as an aid in clinical diagnosis of HCV-related infections.

SUMMARY AND EXPLANATION OF THE TEST

The UBI[®] HCV EIA is an immunoassay which employs synthetic peptide antigens derived from HCV epitopes for the detection of antibodies to HCV in human serum or plasma. These antigens, which bind antibodies specific to highly antigenic segments of both the structural and non-structural proteins of the hepatitis C virus, constitute the solid phase antigenic adsorbent.

Specimens with absorbance values greater than or equal to the Cut-off Value are defined as initially reactive. Initially reactive specimens should be retested in duplicate. Specimens which do not react in either of the duplicate repeat tests are considered nonreactive for antibodies to HCV. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatably reactive for antibodies to HCV.

CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The UBI[®] HCV EIA employs an immunosorbent bound to wells of the microplate consisting of synthetic peptides that capture antibodies with specificities for highly antigenic segments of core, NS3, NS4 and NS5 proteins of the Hepatitis C Virus. During the course of the assay, diluted controls and diluted specimens are added to the wells and incubated. HCV-specific antibodies, if present, will bind to the immunosorbent. After a thorough washing of the wells to remove unbound antibodies and other serum components, a standardized preparation of horseradish peroxidase-conjugated goat antibodies specific for human IgG is added to each well. This conjugate preparation is then allowed to react with the captured antibodies. After another thorough washing of the wells to remove unbound horseradish peroxidase-conjugated antibody, a substrate solution containing hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine (TMB) is added. A blue color develops in proportion to the amount of HCV-specific antibodies present, if any, in the serum or plasma samples tested. This enzyme-substrate reaction is terminated by the addition of a solution of sulfuric acid which produces a yellow color. The color intensities that have occurred in each well are then measured spectrophotometrically at a wavelength of 450 nm.

REAGENT COMPONENTS AND THEIR STORAGE CONDITIONS

UBI® HCV EIA	192 tests	576 tests
HCV Reaction Microplates Each microplate well contains adsorbed antigenic HCV synthetic peptides. Store at 2-8°C sealed with desiccant.	2 plates	6 plates
Non-Reactive Control Inactivated normal human serum containing 0.1% sodium azide and 0.02% gentamycin as preservatives. Store at 2-8°C.	1 mL	1 mL
HCV Strongly Reactive Control Inactivated human serum that has a high titer of antibodies with specificity for HCV peptide antigens. Non-Reactive for Hepatitis B surface antigen, HIV-1/2 and HTLV-I antibodies. Contains preservatives: 0.1% sodium azide and 0.02% gentamycin. Store at 2-8°C.	1 mL	1 mL
Specimen Diluent (Buffer I) Phosphate buffered saline solution containing surfactant, heat-treated normal goat serum, casein, bovine serum albumin and preservatives: 0.1% sodium azide and 0.02% cinnamaldehyde. Store at 2-8°C.	45 mL	130 mL
Conjugate Horseradish peroxidase-conjugated goat anti-human IgG antibodies, with 0.02% gentamycin and 0.05% 4-dimethylaminoantipyrine. Store at 2-8°C.	0.4 mL	0.8 mL
Conjugate Diluent (Buffer II) Phosphate buffered saline solution containing surfactant and heat-treated normal goat serum, with 0.02% gentamycin as a preservative. Store at 2-8°C.	30 mL	80 mL
TMB Buffer 3,3',5,5'-tetramethylbenzidine (TMB) solution. Store at 2-8°C.	14 mL	40 mL
Substrate Diluent Citrate buffer containing hydrogen peroxide. Store at 2-8°C.	14 mL	40 mL
Stop Solution Diluted sulfuric acid solution (1.0M H ₂ SO ₄). Store at 2-30°C.	25 mL	80 mL
Wash Buffer Concentrate A 25X concentrate of phosphate buffered saline with surfactant. Store at 2-30°C.	150 mL	3x150 mL
Plate Covers (OPTIONAL, IF INCUBATING IN A CONTROLLED HUMID ENVIRONMENT) Clear, plastic adhesive sheets to be used to cover the Reaction Microplate wells during each incubation. Plastic sheets may be cut, before removing the paper backing, whenever less than a full plate of Reaction Microplate wells is being assayed. Alternatively, standard microplate lids may be used.	6 sheets	18 sheets

MATERIALS REQUIRED - NOT SUPPLIED

1. Manual or automatic multi-channel- 8 or 12 channel pipettors (50 μ L to 300 μ L).
2. Manual or automatic variable pipettors (From 1 μ L to 200 μ L).
3. Incubator ($37 \pm 2^\circ\text{C}$).
4. Polypropylene or glass containers (25 mL capacity), preferably with a cap.
5. Sodium hypochlorite solution, 5.25% (liquid household bleach).
6. The UBI[®] HCV EIA procedure requires a microplate reader capable of transmitting light at a wavelength of 450 ± 2 nm.
7. Aspiration-wash system capable of dispensing and aspirating 250-350 μ L.
8. Pipettor troughs or boats.
9. Reagent grade (USP or equivalent) water.
10. Disposable gloves.
11. Timer.
12. Non-metallic forceps.
13. Absorbent tissue.
14. Biohazardous waste containers.
15. Pipettor tips.
16. Yellow dilution microplate for predilution of specimen samples of performing procedure II. Your product distributor will supply upon request.

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE

1. HANDLE ASSAY SPECIMENS, REACTIVE AND NON-REACTIVE CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT. Wear disposable gloves throughout the test procedure. Dispose of gloves as biohazardous waste. Wash hands thoroughly afterwards.
2. DO NOT SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER. CONJUGATE and REACTION MICROPLATES are matched for optimal performance. Use only the reagents supplied by manufacturer.
3. Do not use kit components beyond their expiration date.
4. Use only reagent grade water to dilute the WASH BUFFER CONCENTRATE.
5. CAUTION:
STOP SOLUTION (1.0 M H₂SO₄) causes burns. Never add water to this product. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
6. Avoid contact of the 1.0 M SULFURIC ACID with any oxidizing agent or metal.
7. Spills should be cleaned thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Iodophor Disinfectant: should be used at a dilution providing at least 100 ppm available iodine. Sodium Hypochlorite:
 - a. Non acid-containing spills should be wiped up thoroughly with a 5.25% sodium hypochlorite solution (liquid household bleach).
 - b. Acid-containing spills should be wiped dry. Spill areas should then be wiped with a 5.25 % sodium hypochlorite solution (liquid household bleach).

8. This product contains sodium azide as a preservative. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of solutions containing sodium azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health (USA) recommends the following: (1) siphon liquid from drain trap using a rubber or plastic hose, (2) fill with 10% sodium hydroxide solution, (3) allow to stand for 16 hours, and (4) flush well with water.

WASTE DISPOSAL

Dispose of all specimens and materials used to perform the test as if they contain infectious agents. The preferred method of disposal is autoclaving for 30 ± 5 minutes at 121°C or higher. Disposable materials may be incinerated. Liquid wastes NOT CONTAINING ACID may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. Allow at least 30 minutes at room temperatures for decontamination to be completed. The liquid may then be disposed of in accordance with local ordinances.

INTERFERING SUBSTANCES

UBI[®] HCV EIA is not affected by hemolysis of specimens. No adverse effects have been noted in the presence of the anticoagulants EDTA, sodium citrate or heparin.

SPECIMEN COLLECTION AND PREPARATION

1. UBI[®] HCV EIA may be performed on human serum or plasma. Specimens containing precipitates or particulate matter may give inconsistent test results. If necessary, specimens should be clarified by centrifugation prior to testing.
2. Specimens must not be heat-inactivated prior to assay.
3. Specimens are not affected by up to three freeze-thaw cycles.

PREPARATION OF REAGENTS

After removing assay reagents from the refrigerator, allow them to reach room temperature and mix thoroughly by gentle swirling before pipetting.

1. WORKING CONJUGATE SOLUTION:

Prepare as step 4 of the ASSAY PROCEDURE. Dilute the conjugate 1:101 with the Conjugate Diluent. Refer to the chart below for the correct amount of Working Conjugate Solution to prepare. Use WITHIN 8 HOURS OF PREPARATION. Use only polypropylene or glass containers. Mix well to ensure a homogenous solution.

WORKING CONJUGATE SOLUTION PREPARATION CHART

Number of Strips	Number of Tests	Conjugate (μL)	Diluent (mL)
1 to 2	8 to 24	25	2.5
3 to 6	25 to 48	50	5.0
7 to 9	49 to 72	75	7.5
10 to 12	73 to 96	100	10.0

2. TMB SUBSTRATE SOLUTION:

Do not prepare in advance. Prepare as step 8 of the ASSAY PROCEDURE. USE WITHIN 15 MINUTES OF PREPARATION, PROTECT FROM DIRECT LIGHT, and KEEP IN A CONTAINER WITH A CAP. Use only polypropylene or glass containers. Mix the TMB Buffer and Substrate Diluent in equal volumes. Refer to the chart below for the correct amount of TMB substrate solution to prepare.

TMB SUBSTRATE SOLUTION PREPARATION CHART

Number of	TMB Buffer	Substrate Diluent
16	1.1	1.1
24	1.6	1.6
32	2.1	2.1
40	2.5	2.5
48	2.5	2.5
56	3.0	3.0
64	3.5	3.5
72	4.0	4.0
80	4.5	4.5
88	4.6	4.6
96	5.0	5.0

3. WASH BUFFER:

Dilute 1 volume of WASH BUFFER CONCENTRATE with 24 volumes of reagent grade water. Mix well. Once prepared, diluted WASH SOLUTION is stable for 3 months with occasional mixing. Store at 2 to 30°C. Do not use diluted WASH SOLUTION until it has reached room temperature (15 to 30°C), if it has been stored in the refrigerator.

4. All materials should be used at room temperature (15 to 30°C). Liquid reagents should be thoroughly and gently mixed before use.

STORAGE INSTRUCTIONS

Opened reagents in their original containers are stable through the expiration date indicated on the label.

1. Store UBI® HCV EIA kit and its components at 2 to 8°C when not in use.
2. Opened, unused strips of the REACTION MICROPLATES must be stored at 2 to 8°C securely sealed in pouch with the desiccant provided. When stored in this manner, the REACTION MICROPLATES are stable for at least 8 weeks after opening.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

1. Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials; do not use reagents which are visibly turbid.
2. The TMB Buffer, Substrate Diluent and the prepared TMB SUBSTRATE SOLUTION should be colorless to pale yellow in color for proper performance of the assay. Any other color may indicate deterioration of the TMB Buffer and/or Substrate Solution.

INSTRUMENTATION

Follow the installation, operation, calibration and maintenance instructions provided by the instrument manufacturers for both microplate reader and automatic microplate washer.

ASSAY PROCEDURE

There are two assay procedures, differing by the method of specimen dilution. Procedure I is designed to accommodate direct pipetting of specimens into the wells using autodilutor. Procedure II allows predilution of specimens. Allow all kit reagents and materials to reach room temperature (15 to 30°C) before use. Do not remove MICROPLATE from the storage bag until needed. Unused strips should be stored at 2 to 8°C securely sealed in the foil pouch with the desiccant provided.

Procedure I (Direct sample addition in reaction microwells)

1. Open the foil pouch and remove the REACTION MICROPLATE. (Do not remove REACTION MICROPLATE from storage until needed.) When not using the entire REACTION MICROPLATE, remove excess strips from the frame and return them to the storage pouch provided and securely seal. Store at 2-8 °C with the desiccant. It may be necessary to replace these strips with blank strips (not provided in the kit) depending on the washing system used.
2. Specimen and Control dilution (1:11): Add 100 µL of Specimen Diluent to all wells. The NON-REACTIVE CONTROL and STRONGLY REACTIVE CONTROL should be assayed in duplicate on each plate with each run of specimens. The NON-REACTIVE CONTROL and STRONGLY REACTIVE CONTROL should be diluted in the same manner as the specimens.
 - A. Add 10 L of Specimen Diluent to wells in duplicate in A1 and B1 as Blank Controls.
 - B. Add 10 L of NON-REACTIVE CONTROL to wells in duplicate in C1 and D1.
 - C. Add 10 L of STRONGLY REACTIVE CONTROL to wells in duplicate in E1 and F1.
 - D. Add 10 L of SPECIMEN to the appropriate wells.Ensure that the contents of the microwells are thoroughly mixed. Manual mixing with a pipette or gently vibrating the plate is acceptable.
3. Cover and incubate 60 ± 2 minutes at $37 \pm 2^\circ\text{C}$.
4. Prepare the WORKING CONJUGATE SOLUTION (1:101) as described in the PREPARATION OF REAGENTS (Section 1) during this incubation prior to washing the REACTION MICROPLATES.
5. Wash the MICROPLATE with WASH BUFFER.
 - A. Automatic Microplate Washer - Use six (6) washes with at least 300 L/well/wash.
 - B. Manual Microplate Washer or Pipettor (8 or 12 channel) - wash six (6) times, using at least 300 µL/well/wash. Fill the entire plate, then aspirate in the same order.
6. Make sure that the rest volume is minimal, e.g. by blotting dry by tapping plate onto absorbent paper.
7. Add 100 µL of the WORKING CONJUGATE SOLUTION (1:101) prepared in step 4 to all wells of the REACTION MICROPLATE. Cover and incubate for 30 ± 1 minutes at $37 \pm 2^\circ\text{C}$.
8. Prepare TMB SUBSTRATE SOLUTION during the incubation prior to use according to the PREPARATION OF REAGENTS (Section 2). Shield the solution from direct light.
9. Repeat the wash procedure as in step 5 and step 6.
10. Add 100 L of the prepared TMB SUBSTRATE SOLUTION to each well of the REACTION MICROPLATE.
11. Cover and incubate for 15 ± 1 minutes at $37 \pm 2^\circ\text{C}$.
12. Add 100 L of STOP SOLUTION to each well of the REACTION MICROPLATE. Mix by gently tapping or vibrating the plate.
13. Read the absorbance at 450 ± 2 nm with air blank. If a dual wavelength measurement is used, the reference wavelength should be selected from 620 nm to 690 nm. NOTE: Absorbance should be read within 15 minutes of the addition of the STOP SOLUTION to the REACTION MICROPLATE.

Procedure II (Sample predilution in the DILUTION MICROPLATE):

1. Separate DILUTION MICROPLATES are provided for specimen dilution when performing tests with this specimen dilution procedure.

To the DILUTION MICROPLATE:

- A. Dispense 200 L of SPECIMEN DILUENT (Buffer I) into all wells.
- B. Add 20 L of CONTROLS in duplicate to wells as stated in Procedure I.
 - a. The NON-REACTIVE CONTROL and STRONGLY REACTIVE CONTROL should be assayed in duplicate on each plate with each run of specimens.
 - b. The NON-REACTIVE CONTROL and STRONGLY REACTIVE CONTROL should be diluted in the same manner as the specimens.
- C. Add 20 L of SPECIMEN to the appropriate wells.

Ensure that the contents of the wells are thoroughly mixed. Manual mixing with a pipette or gently vibrating are acceptable.

2. Open the foil pouch and remove the REACTION MICROPLATE. (Do not remove REACTION MICROPLATE from storage until needed.) When not using the complete REACTION MICROPLATE, remove excess strips from the frame and return them to the storage pouch provided and securely seal. Store at 2-8 °C with the desiccant. It may be necessary to replace these strips with blank strips (not provided in the kit, depending on the washing system used). Transfer 100 L of the CONTROLS and DILUTED SPECIMENS from each well of the DILUTION MICROPLATE to its corresponding well in the REACTION MICROPLATE.
3. Cover and incubate for 60 ± 2 minutes at $37 \pm 2^\circ\text{C}$.
Proceed as in Procedure I, step 4.

RESULTS

The presence or absence of antibody specific for HCV is determined by relating the absorbance of the specimens to the Cut-off Value.

For the assay to be valid, the absorbance difference between the means of the STRONGLY REACTIVE and NON-REACTIVE CONTROLS (SRC - NRC) should be 0.400 or greater. Blank values are for reference only. They should be less than 0.100 absorbance units.

Note: The STRONGLY REACTIVE CONTROL values should be below the upper limit of the linear range of the microplate reader. If they are not, your instrument should be recalibrated and the assays repeated.

Calculation of Results

1. Determine the Mean of the NON-REACTIVE CONTROLS (NRC)

Example:	Non-reactive Controls Absorbance	
		0.090
		0.082
	Total	0.172
	Mean	$0.172/2 = 0.086$

Individual NRC values must be less than or equal to 0.200 absorbance units.

2. Determine the Mean of the STRONGLY REACTIVE CONTROLS (SRC)

Example:	Strongly Reactive Controls	Absorbance
		1.345
		1.391
	Total	2.736
	Mean	$2.736/2 = 1.368$

Individual SRC absorbances should be greater than 0.500 and must be within the range 0.5 to 1.5 times the SRC MEAN.

3. Calculation of Mean SRC-NRC:

For the run to be valid, the mean SRC-NRC must be ≥ 0.400 . If not, improper technique or reagent deterioration may be suspected. Inspect the reagents and repeat the run.

Example:	Mean NRC	= 0.086
	Mean SRC	= 1.368
	Mean SRC-NRC	= $1.368 - 0.086 = 1.282$
		Valid test

4. Calculation of the Cut-off Value:

$$\text{Cut-off Value} = 0.2 \times \text{Mean SRC}$$

Example:	Mean SRC	= 1.368
	Cut-off Value	= $0.2 \times 1.368 = 0.274$

INTERPRETATION OF RESULTS

1. Specimens with absorbance values less than the Cut-off Value are considered nonreactive by the criteria of the UBI[®] HCV EIA and may be considered negative for antibodies to HCV. Further testing is not required.
2. Specimens with absorbance values greater than or equal to the Cut-off Value are considered initially reactive. These specimens should be retested in duplicate (using the original sample) before final confirmation of the result.
3. Initially reactive specimens which do not react in either of the duplicate repeat tests are considered negative for antibodies to HCV. Further testing is not required.
4. Initially reactive specimens which are reactive in one or both of the repeat tests are considered repeatably reactive for antibodies to HCV.
5. Specimens which have been found repeatably reactive are interpreted to be positive for the presence of antibodies to HCV. In most settings, it is appropriate to investigate repeatably reactive specimens by additional more specific tests such as immunoblot assay, peptide-based neutralization EIA or other test method that is capable of identifying antibodies or antigens for specific gene products of HCV.

LIMITATIONS OF THE PROCEDURE

The UBI[®] HCV PROCEDURE and the INTERPRETATION OF RESULTS sections must be closely adhered to when testing for the presence of antibodies to HCV in plasma or serum from individual subjects. Because the UBI[®] HCV EIA was designed to test individual units of serum or plasma, data regarding its interpretation were derived from testing individual samples. Insufficient data are available to interpret tests performed on other bodily fluids, pooled serum or plasma or processed plasma, and products made from such pools. Testing of these specimens is not recommended.

A person whose serum or plasma is found to react in both the EIA and in an additional more specific test for antibodies to HCV or antigen is presumed to be infected with the virus. Appropriate counseling and medical

evaluation should be offered. Such an evaluation should be considered an important part of HCV antibody testing and should include test result confirmation from a freshly drawn sample.

Viral hepatitis is a clinical syndrome caused by several etiologic agents and its diagnosis can only be established clinically. UBI[®] HCV EIA\ testing alone cannot be used to diagnose these conditions, even if the recommended investigation of reactive specimens confirms the presence of HCV antibodies. A negative test result at any point in the serologic investigation does not preclude the possibility of exposure to or infection with HCV.

UBI® is a registered trademark of United Biomedical, Inc.

Manufactured for:

United Biomedical, Inc.

25 Davids Drive

Hauppauge, NY 11788

U.S.A.

U.S.A. Patents 5,106,726; 5,436,126; 5,747,239; 5,582,968;
5,639,594; 5,736,321; Australia 635,124; 646,275; Canada 2,036,463; 2,047,792; Europe (EPO) 442,394B1;
468,527B1; Japan 3,199,995; Korea 74,862; 70,945; Germany 19,500,394; 19,540,105; 19,549,390;
Netherlands 1,002,149; 1,005,557; 1,005,556; Singapore 49,183; Taiwan 110,771; 89,412; United Kingdom
2,294,690B; and Patents pending

Version 1 (**DI090902**)