Hepatitis C virus Ab ELISA Kit

Catalog Number KA2534

96 assays

Version: 22

Intended for research use only

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Introduction

Intended Use

The Hepatitis C virus Ab ELISA Kit is qualitative detection and screening assay of Antibody to Hepatitis C virus (anti-HCV) in human serum or plasma.

Principle of the Assay

This kit adopts the "direct sandwich principle" as the basis for the assay to detect antibodies to Hepatitis C virus (anti-HCV). It is a enzyme immunoassay kit, which uses recombinant HCV antigens (Core, NS3 and NS5 antigens) for the detection of Antibody to Hepatitis C virus (anti-HCV) in human serum or plasma.[1-3] These antigens, which are reactive with the predominant antibodies of HCV, constitute the solid phase antigenic absorbent. When human serum or plasma is added to the well, the HCV antigens and Anti-HCV will form complexes on the wells if Anti-HCV is present in the specimen. The wells are washed to remove the unbound materials. The diluted HCV Ag•HRPO Conjugate is added to the well and results in the formation of (HCV Ag) • (Anti-HCV) • (HCV Ag•HRPO) complex. After washing out the unbound conjugate, TMB substrate solution is added for color development. The intensity of color development is proportional to the amount of antibodies present in the specimen.-The reaction processes are summarized as follows:

A. Specimen (containing Anti-HCV):

1. Plate (HCV Antigens) + Specimen (containing Anti-HCV) → plate (HCV Antigen) • Anti-HCV
2. Wash to remove the unbound materials.
3. Plate (HCV Antigen)•Anti-HCV + HCV Ag•HRPO → Plate (HCV Antigen)•Anti-HCV•HCV Ag•HRPO complex
4. Wash to remove the unbound materials
5. Plate (HCV Antigen) • Anti-HCV • HCV Ag • HRPO complex + TMB Solution → light blue to blue color
6. Light blue to blue color + 1 N H_2SO_4 → light yellow to yellow color, measured at 450 nm with a selected reference wavelength within 620 to 690 nm [*4]

B. Specimen (without human Anti-HCV):

1. Plate (HCV Antigens) + Specimen (without Anti-HCV) → plate (HCV Antigen)
2. Wash to remove the unbound materials
3. Plate (HCV Antigen) + HCV Ag • HRPO → plate (HCV Antigen)------ No complex will form
4. Wash to remove the unbound materials
5. Plate (HCV Antigen) + TMB Solution (colorless) → colorless
6. colorless + 1 N H_2SO_4 → colorless, measured at 450 nm with a selected reference wavelength within 620 to 690 nm[*4]

General Information

Materials Supplied

List of component

Component	Description	Amount
HCV Antigens Plate	Microtiter plate coated with HCV antigens.	1 plate
Conc. HCV Ag•HRPO Conjugate	Contained HCV Ag・Peroxidase (Horseradish) in buffer with Bovine serum. Preservatives: 0.005 % Sodium azide and 0.05 % Enzyme stabilizer.	1.8 mL
Anti-HCV Positive Control	Inactivated human plasma positive for Anti-HCV. Preservative: 0.099% Sodium azide.	2 mL
HC Negative Control	Normal human plasma non-reactive for Antibody to HCV. Preservative: 0.099% Sodium azide.	3 mL
Conjugate Diluent	PB-buffer with Bovine serum and Tween-20. Preservatives: 0.005 % Sodium azide and 0.05 % Enzyme stabilizer.	24 mL
TMB Substrate Solution A	3, 3', 5, 5'-tetramethylbenzidine (TMB) in an organic base.	12 mL
TMB Substrate Solution B	Acetate acid buffer with Urea Hydrogen Peroxidase.	12 mL
Conc. Washing Solution D (20X)	Phosphate buffer with Tween-20.	110 mL
Stop Solution	1 N Sulfuric Acid	12 mL

Accessories: (provided as needed)

Items
Adhesive slips
Absorbent pads
Black cover

Storage Instruction

- ✓ The kit must be stored at 2 to 8°C. Do not freeze.
- ✓ Strips of the plate should be used within one month once the original aluminum foil bag is opened. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
- ✓ Return reagents to 2 to 8°C immediately after use.
- ✓ Washing Solution D (20X) Concentrate can be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in 37°C water bath till crystal dissolved.
- ✓ Washing Solution D (20x) and 1 N H_2SO_4 can be stored at +2 to +30°C. The other reagents should be refrigerated at 2 to 8°C.

Kit/components	Storage temp.	State	Stability
Hepatitis C virus Ab ELISA Kit	2 - 8°C	Original	18 months
		Once open	1 month
Anti-HCV Positive Control	2 - 8°C	Original	18 months
		Once open	1 month
HC Negative Control	2 - 8°C	Original	18 months
		Once open	1 month
HCV Antigens Plate	2 - 8°C	Original	18 months
		Once open	2 month
Conc. HCV Ag•HRPO Conjugate Solution	2 - 8°C	Original	18 months
		Once open	1 month
Diluted HCV Ag•HRPO Conjugate Solution	Room Temp.	Diluted	6 hours
	2 - 8°C	Diluted	2 days
Conjugate Diluent	2 - 8°C	Original	18 months
		Once open	1 month
Washing Solution D Concentrate (20X)	Room temp.	Original	24 months
		Once open	1 month
20X Diluted Washing Solution	Room temp.	Diluted	2 days
	2 - 8°C	Diluted	1 week
TMB Substrate Solution A	2 - 8°C	Original	24 months
		Once open	1 month
TMB Substrate Solution B	2 - 8°C	Original	24 months
		Once open	1 month
TMB Substrate Solution Mixture	Room temp.	Mixture	6 hours
Stop Solution	Room temp.	Original	24 months
		Once open	1 month

Materials Required but Not Supplied

- ✓ 50 µL, 100 µL, 200 µL and 1 mL micropipettes and tips are needed.
- ✓ Water-bath or incubator.
- ✓ Tubes for specimen dilution.
- ✓ Plate washing equipment.
- ✓ ELISA microwell reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength[*4], bandwidth 10 nm.
- ✓ Purified water: distilled or deionized water.
- ✓ Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

- ✓ Limitations and interferences:
  - This reagent kit is to be used for un-pooled human serum or plasma only.
  - Specimens with very low level of Anti-HCV may not consistently repeat positive. In this case, it is recommended to test follow-up samples.
  - Anti-HCV negative result does not preclude the possibility of infection with HCV.
  - Non-repeatable false positive results may occur due to non-specific binding of the sample and conjugate to the wall of the well(s).
  - Potential Interfering Substances: there is no significant influence on Hepatitis C virus Ab ELISA Kit.

Precautions for Use

- ✓ For professional use only.
- ✓ This reagent kit is for research use only.
- ✓ Bring all kit reagents and samples to room temperature (20 to 30°C) and mix gently before use.
- ✓ Do not use reagent past its expiration date.
- ✓ Do not interchange reagents between different lots.
- ✓ Do not pipette in the mouth.
- ✓ Do not smoke or eat in areas where specimens or reagents are handled.
- ✓ All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols.
- ✓ Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
- ✓ Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:
  Both liquid and solid waste should be autoclaved maintaining 121°C for at 30 minutes.
  Solid waste can also be incinerated.
  Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
- ✓ 1 N Sulfuric Acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 1 N sulfuric acid with skin and mucous membranes. In case of contact, flush immediately with abundant amounts of water.
  In case of inhalation, find fresh air immediately and seek medical advice in case of pain.
- ✓ TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
- ✓ Although all human sourced material, such as Anti-HCV Positive Control and HC Negative Control , are tested free from HBsAg and Anti-HIV and inactivated at 56°C for one hour, the reagent should still be handled as potential infectious material[*5].

Assay Protocol

Reagent Preparation

✓ Plate Washing Procedure

- Preparation of washing solution:
  Dilute Washing Solution D (20X) concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
- Plate washing:
  For plate washer with overflow aspirating function: 6 cycles with at least 0.5 mL washing buffer per well per cycle.
  Or
  For plate washer without overflow aspirating function: 8 cycles with at least 0.35 mL washing buffer per well per cycle.
- Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

WARNING: Improper washing will cause false results.

Sample Preparation

✓ Specimen Collection and Storage

- Either serum or plasma can be used with this kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
- Specimens must be stored at 2 to 8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
- Frozen specimens must be thoroughly thawed and mixed homogenously before test.
- Avoid multiple freeze-thaw procedures.
  Note: Incompletely coagulated sera and microbial-contaminated specimens should not be used.

Assay Procedure

1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to 37±1°C.
2. Preparation of Diluted Conjugate
   ✓ Use only clean container to avoid contamination.

- ✓ Prepare diluted conjugate by making 1:20 dilution of Conc. HCV Ag•HRPO conjugate with conjugate diluent, or following Conjugate Preparation Chart below. Swirl gently to mix thoroughly and avoid foaming.
- ✓ Excess diluted conjugate solution should be discarded after use.
- ✓ Conjugate Preparation Chart:

Number of wells used	Volume of Conjugate Diluent needed (mL)	Volume of Conc. HCV Ag• HRPO conjugate needed (µL)
8	1	50
16	2	100
24	3	150
32	4	200
40	5	250
48	6	300
56	7	350
64	8	400
72-80	9	450
81-96	10	500

3. Reserve one well for Blank. Do not add any specimen or specimen diluent into the well for blank.
4. Prepare the needed number of wells, including 1 well for Blank, 3 wells for Negative Control, 2 wells for Positive Control, and 1 well for each Specimen.
5. Sample input:
   - ✓ Add 100 µL of Positive Control, Negative Control and specimen to each appropriate well of HCV Antigens Plate.
   - ✓ Mix well by tapping the plate gently.

   NOTE: Use a new pipette tip after each sampling to avoid cross-contamination.
6. Seal the Plate with an Adhesive Slip.
7. Incubate the plate in a 37±1°C water bath or circulative incubator for 60 minutes.

   NOTE: Do not stack plates.
8. At the end of the incubation period, remove carefully the adhesive slip and discard.
9. Wash the plate according to Plate Washing Procedure.
10. Add 100 µL of the Diluted Conjugate in each well, except the blank.
11. Seal the plate with an Adhesive Slip.
12. Incubate the Plate in a 37±1°C water bath or circulative incubator for 30 minutes.
13. Repeat step 8 and 9.
14. Select one of the following methods for color development:

    A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µL of the mixture solution to each well including the blank.

B. Add 50 µL of TMB Substrate Solution A first, and then add 50 µL of TMB Substrate Solution B into each well including the blank. Carefully mix well.

NOTE: TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 6 hours after mix. The mixture should be avoided from intense light.

15. Seal the plate with an Adhesive slips and incubate at 37±1°C for 15 minutes.
16. Stop the reaction by adding 100 µL of Stop Solution to each well including the blank.
17. Determine the absorbance of Controls and test specimens within 15 minutes, measured at 450 nm with a selected reference wavelength within 620 to 690 nm[*4].

    Use the blank well to blank the spectrophotomer.

NOTE: The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid. Substrate blank: absorbance value must be less than 0.100.

Data Analysis

Calculation of Results

1. Calculation of the NCx (Mean Absorbance of Negative Control).

   Example:

Sample No.	Absorbance
1	0.045
2	0.060
3	0.051

   NCx = (0.045+0.060+0.051) / 3 = 0.052

   NCx must ≤ 0.200, otherwise the test is invalid.

2. Calculation of PCx (Mean Absorbance of Positive Control)

   Example:

Sample No.	Absorbance
1	1.510
2	1.826

   PCx = (1.510 + 1.826) / 2 = 1.668

   PCx must ≥ 0.600, otherwise the test is invalid.

3. Calculation of P-N Value

   P-N = PCx - NCx

   Example:

   P - N = 1.668 – 0.052= 1.616

   P-N value must ≥ 0.400, otherwise the test is invalid.

4. Calculation of the Cutoff Value

   Cutoff Value = NCx + 0.100

   Example:

   Cutoff Value = 0.052 + 0.100 = 0.152

5. Calculate the cut-off index of the specimens

   Cutoff Index = Sample OD Value / Cutoff Value

   Example:

   Sample Value is 0.596

   Cutoff Index = 0.596 / 0.152 = 3.921

6. Gray Zone: Cut-off index = 1.000 ～ 1.500

- Quality Control of the Test Run
  - ✓ NCx must be ≤ 0.200, otherwise the test is invalid.
  - ✓ PCx must be ≥ 0.600, otherwise the test is invalid.
  - ✓ P-N Value must be ≥ 0.400, otherwise the test is invalid.

    NOTE: Negative Control: absorbance value must be less than or equal to 0.200 after subtracting the blank.

✓ Result Interpretation

1. Specimens with CUTOFF INDEX < 1.000 are considered NON-REACTIVE
2. Specimens with CUTOFF INDEX ≥ 1.000 are considered as initially REACTIVE. They should be RETESTED in duplicate.

   If both CUTOFF INDEXES of the duplicate are GREATER than 1.500 the specimen is considered to be repeatedly REACTIVE for Anti-HCV by the criteria of Hepatitis C virus Ab ELISA Kit.

   Specimens repeatedly reactive in the Hepatitis C virus Ab ELISA Kit should be further tested by additional, more specific tests.
3. Initially reactive specimens, of which both CUTOFF INDEXES of the duplicate retest are LESS than 1.000, will be considered NON-REACTIVE for Anti-HCV.
4. If one of the two CUTOFF INDEXES of the duplicate is GREATER than 1.000 but LESS than 1.500, the specimen may be interpreted as QUESTIONABLE and this individual should be monitored in follow up samples, or additional more specific tests should be used.
5. If one of the CUTOFF INDEX of the duplicate is GREATER than 1.500 and the other one is LESS than 1.000, this indicates unusual experimental error. The test should be repeated again.

Performance Characteristics

- Analytical Specificity

  Potential Interfering Substances: There is no significant influence on Hepatitis C virus Ab ELISA Kit.

Potential Interfering Substances	n tests	n reactive	n non-reactive
Serum with interfering substances in fixed ratios (Triglycerides, hemoglobin, bilirubin, monoclonal IgG and IgM, and rheumatoid factor)	50 tests	0	50
Inhibition panels (EDTA, hemoglobin, triglyceride, bilirubin, and heparin)	14 negative samples	0	14
	14 positive samples	14	0
Anticoagulant Panels (Serum, EDTA plasma, heparinized plasma, and citrated plasma)	25 negative samples	0	25
	25 positive samples	25	0
Total	128 tested samples	39	89

Note: The concentration of triglycerides, hemoglobin, bilirubin, and rheumatoid factor are up to 2.256 mg/dL, 5.11 g/L, 29.11 mg/dL, and 1.558 IU/mL separately.

- Specificity

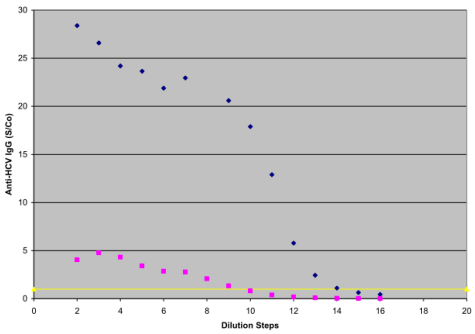
  Specificity = 5356/5369 = 99.8 %

Potential Interfering Substances	n tests	n reactive	n non-reactive	Specificity
Blood donors	5169	12	5157	99.77 %
Hospital specimens	200	1	199	99.5 %
Potentially cross-reacting serum / plasma specimens	100	0	100	100%
Total	5469	13	5456	99.8 %

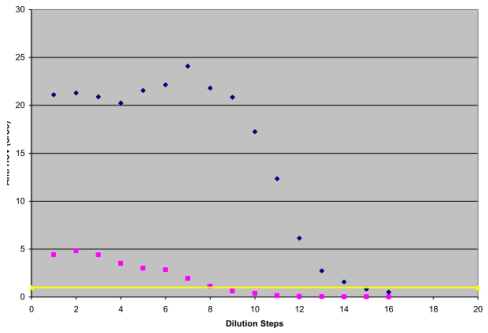
- Analytical Sensitivity

  The analytical sensitivity of Hepatitis C virus Ab ELISA Kit assay is higher than the comparison test.

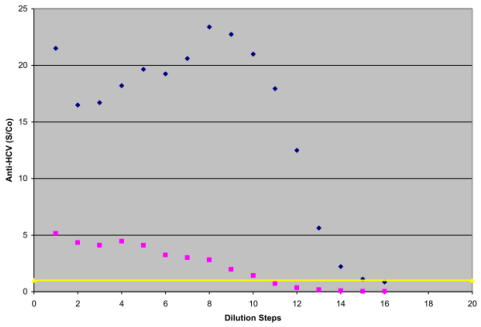
Analytical Sensitivity PEI Reference Material



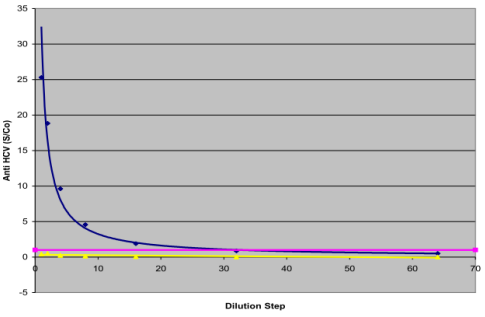
Serial Dilution Positive Sample 1



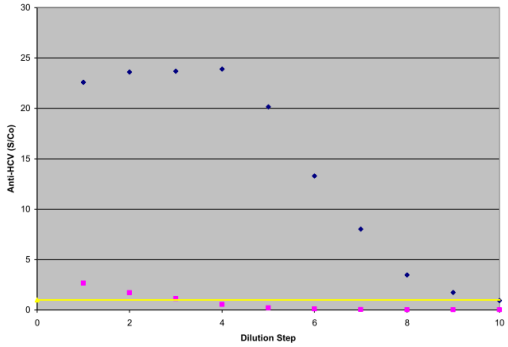
Serial Dilution Positive Sample 2

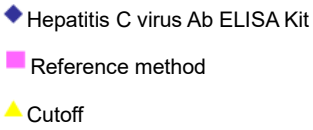


Serial Dilution of the Positive Control



PeliCheck Anti-HCV Reference Panel



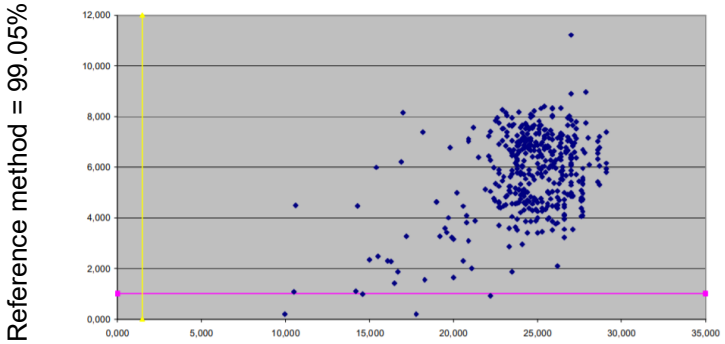


- Sensitivity

  HCV infected individuals:

  The sensitivity of the Hepatitis C virus Ab ELISA Kit was determined to be 100 %. 421 of 421 positive samples including 20 samples per genotype for genotypes 1a – 4a and 5 samples for genotype 6 were tested and confirmed reactive for HCV antibodies.

Total Sensitivity n=421



Hepatitis C virus Ab ELISA Kit = 100 %

- Precision

  ✓ Intra-assay Reproducibility

CUTOFF INDEX	Positive Serum 1		Positive Serum 2		Positive Control	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Day 1	4.49	7.22	9.01	5.94	21.79	4.91
Day 2	4.16	13.06	8.54	9.41	21.44	4.23
Day 3	5.07	5.24	10.64	9.27	21.70	3.08
Mean	4.57	8.51	9.40	8.21	21.64	4.07

  ✓

✓ Total Imprecision

Lot C68332PT		CUTOFF INDEX			
Run-No	Run Date	NC	PS1	PS2	PC
1	15/7/08	0.31	5.03	10.70	22.50
2	18/7/08	0.30	4.52	7.83	23.56
3	18/7/08	0.30	4.83	9.58	21.16
4	21/7/08	0.29	5.72	10.40	21.38
5	22/7/08	0.33	4.91	8.82	20.72
6	22/7/08	0.33	4.53	10.10	17.25
7	23/7/08	0.27	3.79	6.39	16.86
8	23/7/08	0.34	4.21	8.08	18.42
9	24/7/08	0.29	5.03	7.96	20.59
10	24/7/08	0.35	4.59	8.48	20.59
MEAN		0.31	4.73	8.83	20.30
SD		0.03	0.52	1.36	2.17
CV		8.34	11.05	15.34	10.69

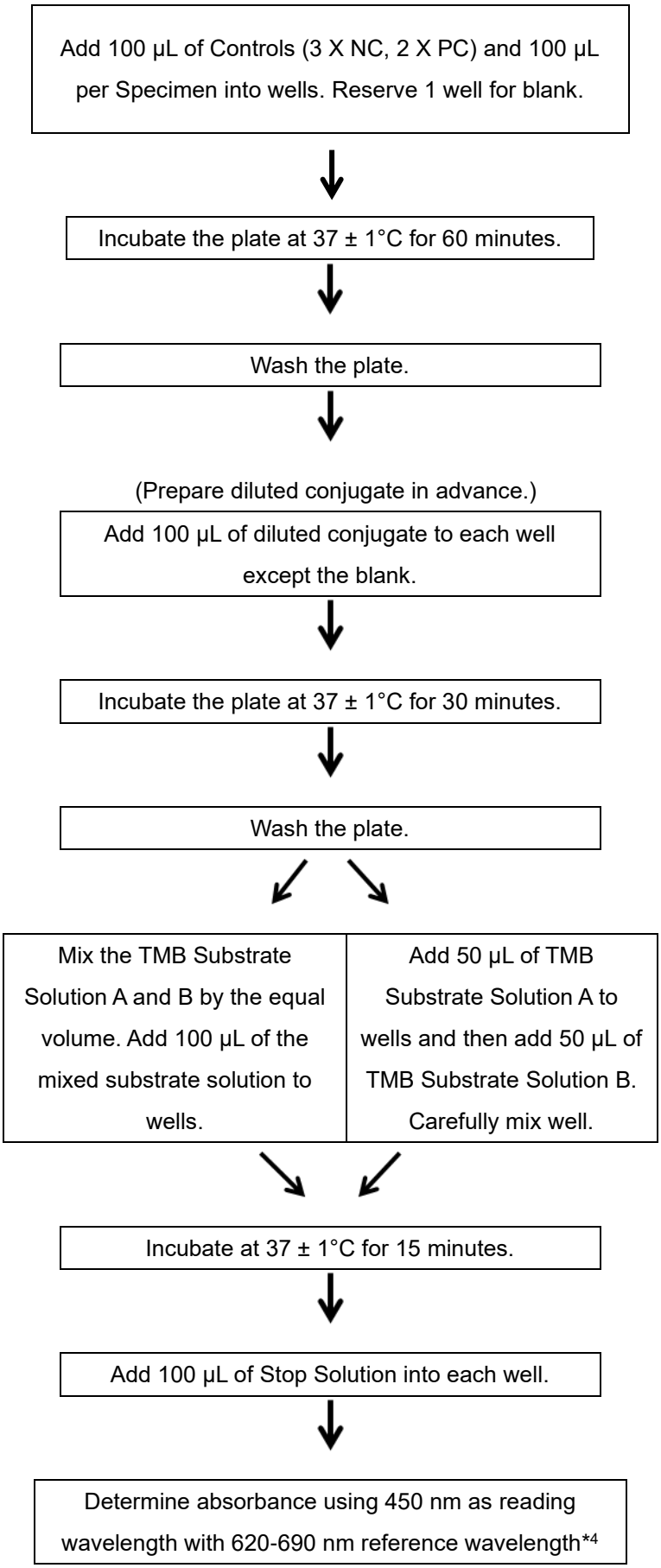
Lot C68332PT		CUTOFF INDEX			
Run-No	Run Date	NC	PS1	PS2	PC
1	19/8/08	0.33	5.63	10.05	20.08
2	19/8/08	0.25	4.96	10.05	18.10
3	20/8/08	0.29	5.62	10.05	21.48
4	21/8/08	0.34	4.05	9.49	20.05
5	21/8/08	0.33	5.31	10.20	19.36
6	22/8/08	0.31	5.34	8.97	19.08
7	22/8/08	0.31	5.39	9.16	19.72
8	25/8/08	0.25	4.31	8.64	18.79
9	25/8/08	0.30	5.74	10.60	21.10
10	26/8/08	0.27	6.03	11.30	20.83
MEAN		0.30	5.15	9.94	19.86
SD		0.03	0.63	0.85	1.07
CV		10.82	12.22	8.51	5.39

PS1: positive serum with Anti-HCV levels borderline

PS2: positive serum with clearly above cutoff value

PC: positive control

✓ Flow chart of the test procedure



Resources

Troubleshooting

If the result cannot be reproduced, perform a preliminary troubleshooting by checking the possibilities listed below:

- ✓ Improper washing procedure.
- ✓ Contamination with positive specimen.
- ✓ Add wrong volume of sample, conjugate or substrates.
- ✓ The well rim is contaminated with conjugate.
- ✓ Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.
- ✓ Wrong incubation time or temperature.
- ✓ Obstructed or partial obstructed washer aspirate/dispense head and needles.
- ✓ Insufficient aspiration.

References

1. Abe K, Inchauspe C, Shikate T, and Prince AM. (1992) Three different patterns of hepatitis C virus infection on chimpanzees. Hepatology, 15:690.
2. Claets H, Volckaerts A, De Beenhouwer H, Vermylen C. (1992) Association of hepatitis C virus carrier state with the occurrence of hepatitis C virus core antibodies. J. Med. Virol. 36:259-264.
3. Beach MJ, et al. (1992) Temporal relationship of hepatitis C virus RNA and antibody responses following experimental infection of chimpanzee. J Med. Virol. 36:226-237.
4. The reference wavelength of spectrometer could be 620nm to 690nm. However, user should validate the spectrometer in combination with this kit before use.
5. Incomplete inactivation of hepatitis B virus after heat treatment at 60°C for 10 hours, J. Infect. Dis. 138:242-244.
6. The supplier is: VQC-AcroMetrix: Jan Steenstraat 1,NL-1816 CT Alkmaar, The Netherlands. Type 7 is available in lyophilised or liquid format. The catalogue numbers are S2233 (lyophilised format) and S2058 (liquid format).
7. National Inst. For Biological Standards & Control (NIBSC), Blabche Lane South Mimms Potters Bar Herts EN6 3QG, UK; Anti-HCV British Working Standard, Product Code: 02/238-002.

Plate Layout

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