DRG® HCV ELISA (EIA-5209)

As of 9 Mar. 2011 rm (Vers. 1.1) **RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

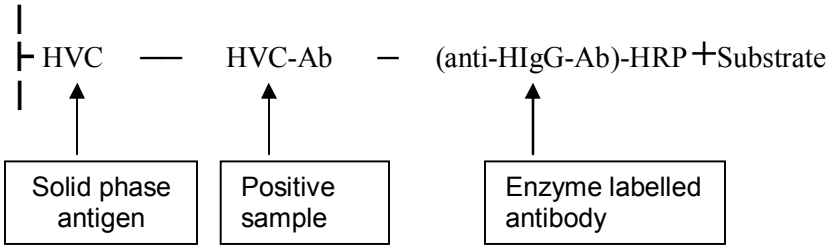
Not for use in diagnostic procedures.

1 INTENDED USE

HCV-ELISA is for the detection of antibodies to Hepatitis C virus in human serum or plasma.

2 PRINCIPLE OF THE ASSAY

The test is an enzyme-immunoassay based on a 'sandwich' principle.



Microplates are coated with HCV specific synthetic antigens derived from "Core" and "NS" regions encoding for conservative immunodominant antigenic determinants (core, NS3, NS4, and NS5).

The solid phase is first treated with the diluted sample and HCV Ab is captured, if present, by the antigens.

After washing out all other components of the sample, in the 2nd incubation bound HCVAb are detected by the addition of anti HIgG antibody, labeled with peroxidase (HRP)

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti HCV antibodies present in the sample.

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3 KITS CONTENTS

	96 tests
1. Coated Microplate Twelve 8-well strips per plate. Each microplate well is coated with HCV specific antigen, and sealed in an aluminum bag containing a silica gel bag as desiccant.	1 plate
2. Negative Control Normal human serum that has non-reactive for HBsAg, antibodies to HCV, and HIV. Contains sodium azide as preservative	1 x 1.0 mL
3. Positive Control Inactivated human serum with high titer antibodies to HCV and non-reactive for HBsAg, and HIV. Contains sodium azide as a preservative	1 x 1.0 mL
4. Specimen Diluent Phosphate buffered saline with Tween-20 containing heat-treated normal goat serum and gelatin. Contains sodium azide as a preservative	1 x 20 mL
5. Conjugate Phosphate buffered saline with Tween-20 containing normal goat serum, protein stabilizer and goat anti-human IgG horseradish peroxidase conjugate. Contains thimerosal as a preservative	2 x 7.5 mL
6. Wash Solution Concentrate (25x). It's buffered saline with Tween-20	1 x 80 mL
7. Chromogen A Contains hydrogen peroxide.	1 x 8 mL
8. Chromogen B Contains tetramethylbenzidine	1 x 8 mL
9. Stopping Solution 2M sulphuric solutions.	1 x 7 mL
10. Plate Covers Cover microplate during incubation.	3 pieces
11. Instructions For Use	1 copy

4 MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled water
2. Manual or automatic pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 200 µL.
3. Pipette tips.
4. Timer

5. Microplate mixer
6. Incubator (37°C).
7. An automatic microplate washer.
8. Microplate reader (equipped with a 450nm and 630nm filter)
9. Gloves

5 SAFETY PRECAUTIONS

Handle assay specimens and controls as if capable of transmitting an infectious agent. Wear disposable gloves throughout the test procedure. Dispose of gloves as biohazard waste. Wash hands thoroughly afterwards.

Do not substitute reagents from one kit lot to another. Controls, conjugate and micro-plates are matched for optimal performance. Use only reagents supplied by the manufacturer.

Do not use kit components beyond their expiration date.

The positive control and negative control should not be diluted.

Use only distilled water.

Allow all kit reagents and materials to reach room temperature before use.

Do not remove micro-plate from storage bag until needed. Unused strips should be stored at 2 °C to 8 °C in its plastic pouch with the desiccant provided.

The sample diluent will change colors when serum or plasma added.

TMB, and peroxide should not come into contact with metals or metal ions since this may give rise to unwanted colour formation.

Avoid contact of Tetramethylbenzidine (TMB) and SULPHURIC ACID with the skin and mucous membranes. If these reagents come into contact with the skin wash with copious amounts of water. Consult physician immediately.

Follow the installation, operation, calibration and maintenance instructions provided by the instrument manufactures for both the micro-plate reader and micro-plate washer.

Incomplete washing will reduce the performance of this kit.

Spills should be cleaned up thoroughly using a 5.25% sodium hypochlorite solution (liquid household bleach).

6 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 half hour at 121 °C or above. Disposable materials may be incinerated.

Liquid wastes not containing acid maybe mixed with sodium hypochlorite in volumes so that the final mixture contains 10% sodium hypochlorite. Allow 30 minutes for decontamination to be completed. Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.

7 STORAGE AND STABILITY

Store the kit and its components at 2 °C to 8 °C when not in use.

Store wash solution concentrate at 2 °C to 8 °C. If wash solution concentrate has been stored for a long time, it may be appear turbid. Performance will not be affected.

Opened, unused strips must be stored with the desiccant provided at 2 °C to 8 °C in the closed pouch.

Indications of deterioration of reagents

Changes in the physical appearance of the reagents supplied may indicate deterioration of these materials. Do not use reagents, which are visibly turbid. Except for specimen diluent and wash solution concentrate.

The substrate should be colorless for proper performance of the assay. Any other colour may indicate deterioration of the substance and/or TMB.

8 INTERFERING SUBSTANCES

This immunoassay is not affected by hemolysis of specimens.
No adverse effects have been noted in the presence of the anticoagulants EDTA, sodium citrate, or heparin.

9 SPECIMEN COLLECTION AND PREPARATION

This immunoassay 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.

Specimens **MUST NOT** be heat inactivated prior to assay.

10 PREPARATION OF REAGENTS

Working substrate solution:

1. It must be prepared just prior to use. Once prepared, solution is stable for 30 minutes at room temperature.
2. Mix the chromogen A and chromogen B thoroughly in a clean bottle before use. This solution will be unstable after 30 minutes. Use immediately after mixing. If small volumes are required, pipette equal volume of chromogen A and chromogen B separately into a clean bottle and mix it. For example, if you require 4 mL of this solution, pipette 2 mL of chromogen A and 2 mL of chromogen B separately into a clean bottle, then mix it completely.

Preparation of the diluted wash solution concentrate:

Dilute 1 volume of wash solution concentrate with 24 volume doubly distilled water. Mix well.

11 PROCEDURE

1. Open the aluminum bag and take out microplate with the required number of strips. The left strips are placed in the plastic pouch along with the desiccant, and sealed (see storage and stability).
2. Set one well as blank, two wells for Negative controls and two wells for Positive controls.
3. Pipette 100 µL of specimen diluent into the sample wells and blank well, excluding the positive and negative controls wells.
   Pipette 100 µL of positive control into each of two positive control wells,
   and 100 µL of negative control into each of two negative control wells respectively.
4. Using pipette, introduce 10 µL of specimen to the assigned sample wells. (Do not add specimen to the blank well!)
5. Seal and incubate for 30 minutes at 37 °C.

6. Wash the micro-plate with wash solution for 5 times. (300 µL/well/wash)!
7. Blot dries by pressing plate onto absorbent tissue.
8. Add 100 µL of conjugate to all wells of the micro-plate.
9. Seal and incubate for 30 minutes at 37 °C.
10. Prepare working substrate solution as described before (Preparation of reagents).
11. Repeat the wash procedure as step 6 and step 7.
12. Add 100 µL of working substrate solution to each micro-well.
13. Seal and incubate for 10 minutes at 37 °C.
14. Add 50 µL of stopping solution to each micro-well, mix gently.
15. Read the absorbance at 450 nm. If a dual filter instrument is used, the reference wavelength should be 620 nm or 630 nm.

    NOTE: Absorbance should be read within 30 minutes of the addition of stopping solution to micro-plate.

12 CALCULATION OF THE RESULTS OF THE SCREENING TEST

All absorbance values, for both the controls and the specimens are subtracted by the value of the blank before interpretation. The presence or absence of antibody specific for HCV is determined by relating the absorbance of the specimens to cut-off value.

Abbreviations

N = the mean absorbance of the negative controls

P = the mean absorbance of the positive controls

S = the absorbance of the test sample

Set the blank well you choose as blank, read the absorbance of the other wells.

13 CALCULATION OF THE CUT OFF-VALUE

The cut-off value is P×10% + N

If P is greater than or equal 2.500, let P equal 2.500.

Test result:

A test is positive if S ≥ cut-off value

A test is negative if S < cut-off value

Checking of test-run validity:

A test-run is only valid if N<0.050 and P>0.800.

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Example: P=1.960 and N=0.012
Cut off Value = P×10% + N
= 1.9600×10% + 0.012 = 0.208

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