DRG® HCV IgM (EIA-3908)

USA: RUO

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INTENDED USE

Enzyme ImmunoAssay (ELISA) for determination of IgM antibodies to Hepatitis C Virus in human plasma and sera.
For research use only.

PRINCIPLE OF THE TEST

Microplates are coated with HCV immunodominant synthetic antigens (core peptide, recombinant NS3, NS4 and NS5 peptides).
In the 1st incubation, the solid phase is treated with diluted samples and anti HCV IgM are captured, if present, by the antigens.
After washing out all the other components of the sample, in the 2nd incubation bound anti-HCV IgM are detected by the addition of anti hIgM 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 IgM antibodies present in the sample.
The presence of IgM in the sample may therefore be quantitated by means of a calibration curve able to determine the content of the antibody in arbU/ml.
Neutralization of IgG anti-HCV, carried out directly in the well, is performed in the assay in order to block interferences due to this class of antibodies in the determination of IgM.

COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. Microplate: **MICROPLATE**
12 strips x 8 microwells coated with HCV-specific synthetic antigens (core, NS4 and NS5 peptides and recombinant NS3). Plates are sealed into a bag with desiccant.
Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Standards: **CAL N° …**
6 x 2.0 ml/vial. Ready to use and color coded standard curve calibrated on an Internal Gold Standard (in absence of a defined international one) or IGS, ranging:
CAL 1 = 0 arbU/ml CAL 2 = 10 arbU/ml
CAL 3 = 25 arbU/ml CAL 4 = 50 arbU/ml
CAL 5 = 100 arbU/ml CAL 6 = 250 arbU/ml.
It contains chemical inactivated HCV IgM positive human plasma, 100 mM Tris buffer pH 7.4±-0.1,
0.2% Tween 20, 0.09% sodium azide and 0.1% Kathon GC as preservatives. The Calibration Curve is coded with blue alimentary dye.
Important Note: Even if plasma has been chemically inactivated, handle this component as potentially infectious.

3. Wash buffer concentrate: **WASHBUF 20x**
1 x 60 ml/bottle, 20x concentrated solution.
Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0±0.2, 0.05% Tween 20 and 0.05% Kathon GC.

4. Enzyme Conjugate: **CONJ**
1 x 16 ml/vial. Ready to use and red colour coded.
It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgM, 5% BSA, 10 mM Tris buffer pH 6.8±0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

5. TMB Substrate Solution: **SUBS TMB**
1 x 16 ml/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
Note: To be stored protected from light as sensitive to strong illumination.

6. Sulphuric Acid: **H_2SO_4 0.3M**
1 x 15 ml/vial. Contains 0.3 M H_2SO_4 solution.
Attention !: Irritant (Xi R36/38; S2/26/30)

7. Specimen Diluent: **DILSPE**
2 x 60 ml/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 ±0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
To be used to dilute the sample.

8. Neutralizing Reagent: **SOLN NEUT**
1 x 8 ml/vial. It contains goat anti hIgG, 2% casein, 10 mM Na-citrate buffer pH 6.0 ±0.1, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

9. Plate sealing foils n°2

10. Package insert n°1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10 µl) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

Warnings and precautions

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.

2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2 - 8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
10. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit did not pointed out any relevant loss of activity up to six 6 uses of the device and up to 6 months.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Sulphuric Acid is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.

2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at +2 °C – 8 °C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20 °C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8 µ filters to clean up the sample for testing.

PREPARATION OF COMPONENTS AND WARNINGS

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storing.
In this case call DRG's customer service.
Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2 °C - 8 °C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Standards
Ready to use components. Mix carefully on vortex before use.

Wash Buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: *Once diluted, the wash solution is stable for 1 week at* +2 °C - 8 °C.

Enzyme Conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
Do not expose to strong illumination, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, possible sterile disposable container.

Sample Diluent
Ready to use component. Mix carefully on vortex before use.

Neutraling Reagent
Ready to use component. Mix carefully on vortex before use.

Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The **ELISA incubator** has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µl/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of ± 5%.
5. The **ELISA microplate reader** has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm;
(b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; repeatability 1%.
Blanking is carried out on the well identified in the section "Internal Quality Control". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an **ELISA automated work station**, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. DRG's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen/Substrate is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Two methods of analysis are possible, as described below:

QUANTITATIVE ASSAY

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples.
2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 µl sample; mix on vortex before use.
   Do not dilute the Standards as they are ready to use.
3. Leave the A1 + B1 wells empty for blanking purposes.
4. Dispense 50 µl Neutralizing Reagent in all the wells, except A1+ B1wells used for blanking operations and the wells used for the Standards.
5. In the identified positions pipette 100 µl of the Calibrators in duplicate followed by 100 µl of diluted samples. Check that Calibrators and samples have been correctly added.
6. Incubate the microplate for **60 min at +37°C**.

   Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*
7. When the first incubation is finished, wash the microwells as previously described (section 9.3)

8. In all the wells, except A1+B1, pipette 100 µl Enzyme Conjugate.
   Incubate the microplate for **60 min at +37°C** .

   Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*
9. When the second incubation is finished, wash the microwells as previously described (section 9.3)
10. Pipette 100 µl Chromogen/Substrate into all the wells, A1 + B1 included.

    Important note: *Do not expose to strong direct light. High background might be generated.*
11. Incubate the microplate protected from light **at room temperature (18-24°C) for 20 minutes** . Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from yellow to blue.
13. Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

QUALITATIVE ASSAY

1. Place the required number of strips in the plastic holder and carefully identify the wells for calibrators and samples.
2. Dilute samples **1:101** dispensing 1 ml Sample Diluent into a disposable tube and then 10 µl sample; mix on vortex before use.
   Do not dilute the Standards as they are ready to use.
3. Leave the A1 well empty for blanking purposes.
4. Dispense 50 µl Neutralizing Reagent in all the wells, except A1 well used for blanking operations and the wells used for the Standards.
5. Then pipette 100 µl of Calibrator 0 arbU/ml in duplicate, 100 µl of Calibrator 10 arbU/ml in duplicate and finally 100 µl of diluted samples. Check that Calibrators and samples have been correctly added.
6. Incubate the microplate for **60 min at +37°C**.

   Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*
7. When the first incubation is finished, wash the microwells as previously described (section 9.3)
8. In all the wells, except A1, pipette 100 µl Enzyme Conjugate.
   Incubate the microplate for **60 min at +37°C**.

   Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*
9. When the second incubation is finished, wash the microwells as previously described (section 9.3)
10. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

    Important note: *Do not expose to strong direct light. High background might be generated.*

11. Incubate the microplate protected from light **at room temperature (18-24°C) for 20 minutes** . Wells dispensed with positive samples and with positive calibrators will turn from clear to blue.
12. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 10 to block the enzymatic reaction. Addition of the stop solution will turn the positive calibrators and the positive samples from yellow to blue.
13. Measure the color intensity of the solution in each well, as described in section 9.5 using a 450 nm filter (reading) and if possible a 620-630 nm filter (background subtraction), blanking the instrument on A1 or B1 or both.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

ASSAY SCHEME

Method	Operations
Neutralizing Reagent	50 µl
Calibrators	100 µl
Samples diluted 1:101	100 µl
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µl
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H_2O_2	100 µl
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 µl
Reading OD	450nm

An example of dispensation scheme in **quantitative** assays is reported below:

Microplate

	1	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	CAL 4	S3										
B	BLK	CAL 4	S4										
C	CAL 1	CAL 5	S5										
D	CAL 1	CAL 5	S6										
E	CAL 2	CAL 6	S7										
F	CAL 2	CAL 6	S8										
G	CAL 3	S1	S9										
H	CAL 3	S2	S10										

Legenda: BLK = Blank CAL = Calibrator S = Sample

An example of dispensation scheme in **qualitative** assays is reported below:

Microplate

	1	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S4											
B	CAL 1	S5											
C	CAL 1	S6											
D	CAL 2	S7											
E	CAL 2	S8											
F	S1	S9											
G	S2	S10											
H	S3	S11											

Legenda: BLK = Blank CAL = Calibrator S = Sample

INTERNAL QUALITY CONTROL

A validation check is carried out on the controls and the calibrator any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

Check	Requirements
Blank well	< 0.100 $OD_{450\ nm}$ value
Calibrator 0 arbU/ml	< 0.200 $OD_{450\ nm}$ value after blanking
Calibrator 10 arbU/ml	$OD_{450\ nm}$ > $OD_{450\ nm}$ CAL 0 arbU/ml + 0.100
Calibrator 250 arbU/ml	3.500 > $OD_{450\ nm}$ > 2.000

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 $OD_{450\ nm}$	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Calibrator 0 arbU/ml > 0.200 $OD_{450\ nm}$ after blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive calibrators instead of the CAL 0 arbU/ml; 4. that no contamination of the Cal 0 arbU/ml, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not gecome contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator 10 arbU/ml < CAL 0 + 0.100	1. that the procedure has been correctly executed; 2. that no mistake has occurred during its distribution 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Calibrator 250 arbU/ml < 2.000 $OD_{450\ nm}$	1. that the procedure has been correctly executed; 2. that no mistake has occurred during its distribution 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.

Calibrator 250 arbU/ml > 3.500 OD $_{450\ nm}$ After blanking	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure; 4. that no contamination of the Cal 250 arbU/ml, or of the wells where this was dispensed, has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.

If any of the above problems has occurred, report the problem to the supervisor for further actions.

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