

**REF**

GD6270 00

HCV IgM

Enzyme immunoassay for the determination of IgM
class antibodies to Hepatitis C Virus in serum or plasma

For research use only

INDICATION

Hepatitis C Virus or HCV is an enveloped RNA virus recently classified in the family of Flaviviridae.

The genome encodes for structural components, a nucleocapsid protein and two envelope glycoproteins, and functional constituents involved in the virus replication and protein processing. The nucleocapsid-encoding region seems to be the most conservative among the isolates obtained all over the world.

HCV accounts for about 95% of hepatitis infections in recipients of blood transfusion and 50% of cases of sporadic NANB hepatitis.

HCV commonly gives origin to asymptomatic hepatitis and chronicity develops in a high number of cases, sometime evolving in severe forms of illness, as hepatocarcinoma.

Antibodies directed to the major immunodominant determinants of the viral proteins are detected in patients infected with HCV, early in the course of infection.

The determination of antibody to HCV has become mandatory in the screening of blood units to prevent post-transfusion hepatitis. It is also currently used to follow up risk individuals and patients under treatment with interferon.

PRINCIPLE OF THE ASSAY

Microtiterplates are coated with HCV specific synthetic antigens from "core", "env" and "ns" regions encoding conservative immunodominant antigenic determinants.

The solid phase is first treated with diluted sample.

After the washing step, the specifically bound antibodies are detected with anti-human IgM antibodies, conjugated to horseradish peroxidase (HRP). A substrate/chromogen solution is added and the intensity of the colour developed by the bound enzyme is proportional to the amount of anti-HCV antibodies in the sample.

The Neutralizing Reagent contains human anti-IgG and blocking antibodies to prevent interferences from Rheumatoid Factor and from the IgG eventually present in the specimen.

KIT CONTENT

1. Reagent A – Microplate

12x8 strips.

8 wells breakable strips, coated with HCV specific synthetic antigens. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. Reagent B1 – Enzymatic Tracer 20x

1 vial of 0.8 ml.

Stabilized proteic buffer solution containing human anti-IgM polyclonal antibody conjugated with Horseradish peroxidase (HRP), 20x concentrated.

3. Reagent B2 – Tracer Diluent

1 vial of 16 ml.

Proteic buffer solution for the dilution of the concentrated tracer; it contains 0.02% Gentamicin sulphate and 0.3% Kathon GC as preservatives.

4. Reagent C – Washing Solution 20x

1 vial of 60 ml.

Concentrated solution to be diluted 1:20 with distilled water. It contains PBS buffer pH 7.4 with detergents and 0.1% Kathon as preservative.

5. Reagent D – Chromogen

1 vial of 8 ml.

Ready to use solution containing Tetramethylbenzidine (TMB) with activators and stabilizers, diluted in phosphate/citrate buffer.

Avoid light exposure.

6. Reagent E – Substrate

1 vial of 8 ml.

Ready to use solution containing Urea peroxide diluted in phosphate/citrate buffer.

7. Reagent F – Stop Solution

1 vial of 16 ml.

Ready to use solution, it contains a mixture of 1 M Hydrochloric and Phosphoric acid.

The reagent is **irritant**: x_i R36/37/38; S(1/2)26-45 Handle with care.

8. Reagent G – Sample Diluent

1 vial of 60 ml.

Proteic solution for sample preparation; it contains a detergent, proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

9. Reagent G1 – Neutralizing Reagent

1 vial of 6 ml.

Proteic buffer solution containing blocking anti human IgG for the neutralization of the Rheumatoid Factor and of the IgG; it contains proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

10. Negative Control

1 vial of 2 ml.

Ready to use prediluted human serum base, not reactive for anti HCV IgM antibodies.

It contains proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

11. Positive Control

1 vial of 2 ml.

Ready to use prediluted human serum base, reactive for anti HCV IgM antibodies.

It contains proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

*It has been treated to inactivate HCV, anyhow, handle with care as **potentially infective**.*

12. Cardboard sealers

2 cardboard sealers to be used to cover the plate during the incubations.

13. Package insert: instruction for use GD6270 00 ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HBsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5% for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. The diluted washing solution can be stored for one week at room temperature or 3 weeks at 2-8 °C.
4. Diluted tracer is stable one week at 2-8 °C, if stored in a disposable sterile container.
5. When preparing chromogen/substrate we recommend the use of plastic disposable containers. The chromogen/substrate solution is stable for 4 hours at room temperature, protected from light.
6. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filters of 450 nm and 620-630 nm.
- Microplate incubator set at 37 (±1) °C.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Either serum or plasma can be used. If the assay is not immediately performed, the samples should be kept at 2-8 °C for one week; otherwise they should be stored at - 20 °C. Avoid repeated freeze-thaw cycles. Samples must not be turbid, lipemic, haemolyzed and microbiologically contaminated.

REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:20 with distilled or ELISA grade water (e.g. 60 ml of Reagent C + 1200 ml of distilled water) and mix carefully before use. It is recommended to store diluted washing solution at room temperature for immediate use.
- **TRACER:** dilute concentrated tracer (Reagent B1) 1:20 with Tracer Diluent (Reagent B2) and mix carefully on vortex.
- **CHROMOGEN/SUBSTRATE:** prepare in disposable plastic container, according to needs, the substrate/chromogen solution by mixing Reagent D with Reagent E in equal volumes.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, controls and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute controls and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and controls at 450 nm. The "blinking" of the instrument is to be carried out in the blank reagent well where only substrate-chromogen and stop solutions are added.

ASSAY SCHEME

- Dilute samples 1:50 with Sample Diluent (e.g.: 10 µl sample + 500 µl of Reagent G).
Do not dilute controls. Carefully mix on vortex before dispensing.
- Follow the scheme:

	HCV antigens coated wells			
	REAGENTS	Blank	Controls	Sample
First incubation	Reagent G1 (Neutralizing Reagent)	-	-	50 µl
	Controls	-	100 µl	-
	Diluted sample	-	-	100 µl
	- Cover the strips with cardboard sealer - Incubate 60 minutes at 37 (± 1) °C			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Second incubation	Diluted tracer	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 60 minutes at 37 (± 1) °C			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Chromogen/Substrate (Reagents D+E)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 20 minutes at room temperature (20-25 °C), avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against Blank at 450 and 620-630 nm			

VALIDITY OF THE ASSAY

The assay is to be considered valid if:

- The OD 450 nm of the blanking well is lower than 0.100. Higher values indicate a chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
- After subtracting the blank, the mean OD 450 nm value for the Negative Control is lower than 0.200. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
- The mean OD 450 nm value of the Positive Control is higher than 0.800. Lower values indicate kit or calibrator decay. Before repeating the assay, check the expiry date of the kit.

CALCULATION OF RESULTS

Calculate the mean values of Negative Control (NC) and Positive Control (PC).

Calculate the cut-off value through the following formula:

$$\text{Cut-off} = \text{Mean NC} + 0.250$$

$$\text{Grey-zone} = \text{cut off} \pm 10\%$$

Calculate the mean OD 450 nm value of the samples.

RESULTS INTERPRETATION

- Samples with OD 450 nm **values lower than cut-off** are to be considered **not reactive** to anti-HCV IgM.
- Samples with an OD **value within or exceeding the upper limit of the grey-zone** are reported as **initially reactive**. The samples should be re-tested in duplicate.
 - Initially reactive samples that do not react in both of duplicate repeat tests are reported as negative for IgM antibody to HCV.
 - Initially reactive samples that are confirmed reactive or grey zone have to be submitted to additional more specific tests (confirmatory tests).
 - Repeatedly reactive samples not confirmed positive are considered false-reactive samples.
- Samples with OD 450 nm **values higher than cut-off** are to be considered **reactive** to anti-HCV IgM. **As some false positivity has been described in the medical literature for HCV testing (ranging 2-7% depending on the screened population), the anti-HCV reactivity in positive samples must be confirmed by a confirmatory test.**

Example of calculation:

Negative Control OD 450 nm mean: 0.050
Positive Control OD 450 nm mean: 1.020
Cut-off = NC + 0.250: 0.300
Grey-zone = Cut-off \pm 10%: 0.270 – 0.330

Sample #1 OD 450 nm value: 0.050 negative
Sample #2 OD 450 nm value: 0.279 grey zone
Sample #3 OD 450 nm value: 1.250 positive

ANALYTICAL PERFORMANCES

Reproducibility

a. Within Run

Within run precision has been determined on 20 replicates of three different samples in the same analytical run. CV values ranging from 8.1 to 14.7% have been found, depending on OD 450 nm values.

b. Between Run

Between run precision has been determined on replicates of three different samples with four different lots. CV values ranging from 1.8 to 13.2% have been found, depending on OD 450 nm values.

Diagnostic sensitivity and specificity

Diagnostic sensitivity and specificity have been determined on a panel of positive samples confirmed by clinical evidence and negative samples from blood donors.

The following results have been found:

Sensitivity: >95%
Specificity: >98%

PRECAUTIONS IN USE

Reagent F is irritant (Xi). Refer to Safety Data Sheet.

The other reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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