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IVD | See external label | 2°C–8°C | Σ 96 tests | REF 1708-12

# AccuDiag™ HCV IgM ELISA

**Cat # 1708-12**

| Test | HCV IgM ELISA |
|---|---|
| Method | ELISA: Enzyme Linked Immunosorbent Assay |
| Principle | Indirect ELISA: Antigen Coated Plate |
| Detection Range | Qualitative Positive; Negative control & Cut off |
| Sample | 10 ul Serum |
| Specificity | 99% |
| Sensitivity | 99% |
| Total Time | ~ 75 min |
| Shelf Life | 12 -18 Months from the manufacturing date |

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

## INTENDED USE

The purpose of the HCV IgM ELISA Test is for clinical lab diagnosis and treatment of patients who are suspected of having a hepatitis C virus infection. This HCV IgM ELISA test is an enzyme-linked immunosorbent assay for qualitative identification of IgM antibodies to hepatitis C virus in human serum/plasma.

## SUMMARY

For more than 50% of patients, HCV develops into chronic hepatitis and has become the principal cause of cirrhosis of the liver and hepatocellular carcinomas. HCV is now accepted as the major agent for non-A and non-B hepatitis transfusion infection. HCV is defined as an envelope, single stranded positive sense RNA (9.5kb) affiliated with the Flaviviridae family. Classification of hepatitis C virus is established through six major genotypes and series of subtypes of HCV.

Blood donor transmissions of hepatitis C virus have notably decreased since screening started in 1990. Three generations of HCV ELISA tests have been established and each generation has resulted in improvement in sensitivity of detecting anti-HCV. 1st generation - limited sensitivity and specificity, using recombinant proteins complementary to NS4 region (c100-3). 2nd generation - noticeable improvement in sensitivity and specificity employing recombinant/synthetic antigens from Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200). A 3rd generation was established because antibodies to NS5 non-structural protein of HCV were being developed by infected individuals. Thus, in addition to NS3 (c200), NS4 (c200), and Core (c22), the 3rd generation tests included antigens from NS5 region. This 3rd generation of tests has improved sensitivity and has lessened the time period (60 days) between infection and the subsequent appearance of antibodies to HCV.

Studies have shown that in a majority of cases, when detectable IgM response to both structural and non-structural HCV antigens occurs, the IgM response happens more frequently to the structural (core) antigen. What can be detected in correctly diagnosing HCV infectivity and viremia in blood is the IgM antibody against HCV core protein. What IgM anti-HCV core level is first associated with is HCV genotype, and then it is secondly identified with liver disease necroinflammatory activity. The manifestation of HCV IgM can be classified into four types: (1) persistent positive; (2) intermittent positive; (3) transient positive; (4) persistent negative. Progressive liver damage is normally indicated by IgM responses types 1 and 2. Patients generally recover if positive type #3. No noteworthy relationship occurred between the ALT HCV IgG serum levels, even though there is an important relationship between the levels of serum HCV IgM and ALT. What does seem connected and has diagnostic importance, is the connection between secretion of HCV IgM core antibodies after OLT and recurrence of HCV-associated liver disease. In order to gauge the extent of progressive liver impairment and in aiding antiviral treatment, it is vitally important and useful for the chronic hepatitis C patients to know the detectable amounts of HCV IgM that are present.

## PRINCIPLE OF THE ASSAY

The principle of the HCV ELISA test involves a two-step incubation procedure in which an indirect ELISA assay for IgM antibodies to HCV is established. Before incubation, recombinant, highly immunoreactive antigens corresponding to the core and non-structural regions of HCV, are pre-coated to the polystyrene microwell strips.

While the first incubation stage takes place, HCV-IgM specific antibodies (if present) will be bound to the solid phase pre-coated HCV antigens. The next step is to wash the wells to remove unbound materials. What must be added next is the rabbit anti-human IgM antibodies (anti-IgM) conjugated to

horseradish peroxidase (HRP-Conjugate). The second incubation stage involves these labeled antibodies, which will be bound to any antigen-IgM complexes previously formed. The following step involves washing the wells to remove any unbound HRP-Conjugate. Next, add to the wells the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. A blue-colored product develops in the presence of the antigen-antibody-anti-IgM (HRP) immunocomplex and when the colorless chromogens are hydrolyzed by the bound HRP conjugate. After stopping the reaction with sulfuric acid, the blue color turns yellow. The color intensity can be gauged proportionally to the amount of antibody captured in the wells, and to the amount in the sample, respectively. The wells remain colorless if the HCV-IgM result is negative.

**Assay principle scheme: Indirect ELISA.**

Ag(p)+Ab(IgM)→[Ag(p)–Ab(IgM)+ENZ]→[Ag(p)–Ab(IgM)–ENZ]→blue→yellow (+)
Ag(p) →[Ag(p) + ENZ]→[Ag(p) ] → no color (-)

| Incubation1 | Incubation 2 | Immobilized Complex | Coloring | Results |
|---|---|---|---|---|
| 30min. | 30min. | | 15min | |

**Ag (p)**–pre-coated HCV antigens (core, NS3/4, NS5);
**Ab(s)**–HCV antibodies in sample (IgM);
**ENZ–**HRP conjugated rabbit anti-human IgM

# COMPONENTS

**96 Tests**

- **MICROWELL PLATE** 1plate
  Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant.
  8×12/12×8-well strips per plate.
  Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately.
  Place unused wells and strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.
- **NEGATIVE CONTROL** 1vial
  Blue-colored liquid filled in a vial with green screw cap.
  0.5 ml per vial.
  Protein-stabilized buffer tested non-reactive for HCV IgM antibodies.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃.
- **POSITIVE CONTROL SERUM** 1vial
  Red liquid filled in a vial with red screw cap.
  0.5 ml per vial.
  HCV IgM antibodies diluted in protein-stabilized buffer.
  Preservatives: 0.1% ProClin 300.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃.
- **SPECIMEN DILUENT** 1vial

Blue-colored liquid filled in a white vial with blue screw cap.
14 ml per vial
Protein-stabilized buffer, casein, and sucrose solution.
Ready to use as supplied.
Once open, stable for one month at 2-8 ℃.

- **HRP-CONJUGATE REAGENT** 1vial
  Red-colored liquid filled in a white vial with red/orange screw cap.
  14 ml per vial.
  Horseradish peroxidase-conjugated rabbit anti-human IgM antibodies.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃.
- **STOCK WASH BUFFER** 1bottle
  **DILUTE BEFORE USE**
  Colorless liquid filled in a clear bottle with a white screw cap.
  50 ml per bottle.
  PH 7.4, 20 × PBS (Contains Tween-20 as a detergent)
  The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8 ℃ .
- **CHROMOGEN SOLUTION A** 1vial
  Colorless liquid filled in a white vial with a green screw cap.
  8 ml per vial.
  Urea peroxide solution.
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃.
- **CHROMOGEN SOLUTION B** 1vial
  Colorless liquid filled in a black vial with a black/brown screw cap.
  8 ml per vial.
  TMB solution (Tetramethyl benzidine dissolved in citric acid)
  Ready to use as supplied.
  Once open, stable for one month at 2-8 ℃.
- **STOP SOLUTION** 1vial
  Colorless liquid filled in a white vial with a yellow/white screw cap.
  8 ml per vial.
  Diluted sulfuric acid solution (2.0M $H_2SO_4$).
  Ready to use as supplied.
- **PLASTIC SEALABLE BAG** 1unit
  For enclosing the strips not in use.
- **CARDBOARD PLATE COVER** 3sheets
  To cover the plates during incubation and prevent evaporation
  Or contamination of the wells.
- **PACKAGE INSERTS** 1copy

## ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.

- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5 ℃.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

## SPECIMEN COLLECTION, TRANSPORTAION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Does not heat inactivate samples? This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

## SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution (final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted 1 **to 20** before use. For one plate, mix 50 ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

## STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C, do not freeze. To assure maximum performance of this HCV IgM ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

# PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** IVD

**FOR PROFESSIONAL USE ONLY**

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8℃ immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so may cause in low sensitivity of the assay.
5. Does not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading?
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121℃ or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M $H_2SO_4$) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.

21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

## ASSAY PROCEDURE

**Step1** **Reagents preparation:** Allow the reagents to reach room temperature **(18-30°C)** for at least 15-30minutes Check the Wash Buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock wash buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the Wash buffer.

**Step2** **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**A1**, Neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

**Step3** **Adding Diluent:** Add **100µl** Specimen Diluent into each well except in the blank and Positive/Negative controls wells.

**Step4** **Adding Sample:** Add **Sample 10µ**l, or Positive control/Negative control, 100µl into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative Control and Positive Control as to avoid cross-contamination.** Mix by tapping the plate gently.

**Step5** **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

**Step6** **Washing:** After the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remaining liquids.

**Step7** **Adding HRP-Conjugate:** Add **100µl** HRP-Conjugate to each well except the Blank.

**Step8** **HRP-Conjugate Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37ºC.**

**Step9** **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash buffer as in **Step 6.**

**Step10** **Coloring:** Dispense **50µl** of Chromogen A and 50µl Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at **37°C for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen A/B solutions produces blue color in Positive control and HCV IgM positive sample wells.

**Step11** **Stopping Reaction:** Using a multichannel pipette or manually add **50µl** Stop Solution into each
well and mix by tapping the plate gently. Intensive yellow color develops in Positive control and HCV IgM positive sample wells.

**Step12** **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results (**Note:** read the absorbance within **5** minutes after stopping the reaction).

## INTERPRETATION OF RESULTS AND QUALITY CONTROLS

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each

sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

**Calculation of Cut-off value (C.O.) = *Nc + 0.12**

***Nc** = the mean absorbance value for three negative controls.
**Important: If the mean OD value of the negative control is lower than 0.02, take it as 0.02.If higher than 0.02 see the Quality Control Range.**

**Example:**
1. Calculation of Nc:

| Well No | B1 | C1 | D1 |
|---|---|---|---|
| Negative controls OD value | 0.014 | 0.012 | 0.016 |

Nc= 0.014
(The mean value is lower than 0.02, so take it as 0.02).
2. Calculation of Cut-off (C.O.) = 0.02 + 0.12 = 0.14

If one of the Negative control values does not meet the Quality
Control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

**2. Quality control range:**
The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

**3. Interpretations of the results:**
(S = the individual absorbance (OD) of each specimen)
**Negative Results (S/C.O. <1)** samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no IgM-class antibodies to hepatitis C virus have been detected with this HCV IgM ELISA kit. The result from this assay should not be used alone to establish the infection state.
**Positive Results (S/C.O.≥1):** samples giving an absorbance greater than, or equal to the Cut-off value are considered initially reactive which indicates that IgM antibodies to hepatitis C virus have been detected using this HCV IgM ELISA kit. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples could be considered positive for HCV IgM. Positive results with HCV IgM detection indicate possible acute or chronic infection with HCV.
**Borderline (S/CO =0.9-1.1):** Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline samples and retesting of these samples in duplicates is recommended. Repeatedly positive samples can be considered positive for HCV IgM.
Follow-up and supplementary testing for any HCV IgM positive samples with other analytical system (e.g. RIBA, WB) is required to establish the infection state.

# TEST PERFORMANCE AND EXPECTED RESULTS

**1. SPECIFICITY:**

| Healthy blood donors | No | - | + | Confirmed positive | False positive | Specificity |
|---|---|---|---|---|---|---|
| *Location 1 | 538 | 532 | 6 | 4 | 2 | 99.62% |
| *Location 2 | 860 | 855 | 5 | 2 | 3 | 99.69% |
| *Location 3 | 278 | 278 | 0 | 0 | 0 | 100% |
| TOTAL | 1676 | 1763 | 11 | 6 | 5 | 99.87% |

The samples were tested during routine blood screening for HCV in three blood banks in China. All initially positive samples were tested with RIBA to confirm the results

**2. SENSITIVITY:**

| Clinical studies | No | + | Reference HCV IgM EIA | Reference HCV IgG EIA | Detected positive rate | Positive agreement with the reference HCV IgM EIA |
|---|---|---|---|---|---|---|
| Acute hep. C | 106 | 65 | 66 | 97 | 68.9% | 98.48% |
| Chronic Hep. C | 151 | 128 | 129 | 151 | 85% | 99.22% |

**Reference assays: third generation HCV IgG EIA and commercially available HCV IgM EIA.**

**DATA FROM IN-HOUSE TESTING:**

| Sample type | Negative Control | Negative sample | Weak positive | Moderate positive | Strong positive | Positive control |
|---|---|---|---|---|---|---|
| S/CO | 0.045 | 0.238 | 1.6 | 5.1 | 9.27 | 11.36 |
| CV% | NA | NA | 8.2% | 5.5% | 4.7% | 4.5% |

# LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The assay is designed to achieve very high sensitivity and specificity performance characteristics and the “indirect model” minimizes the unspecific reactions, which can occur due to interference between unknown meters in sample and the rabbit anti-human IgM used as a conjugate. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.

3. Positive results must be confirmed with another available method. Any positive result must be interpreted together with the patient clinical information and other laboratory results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values. False negative results can occur from inhibition of specific IgM in the presence of high titers of specific IgG. The removal of IgG can be helpful to prevent false negative results and methods for this are given elsewhere.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results can not be used to measure antibodies concentrations.

## INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

## VALIDITY

**Please do not use this kit beyond the expiration indicated on the kit box and reagent labels.**

| **SUMMARY OF THE ASSAY PROCEDURE:** | |
|---|---|
| **Add sample diluent** | **100µl** |
| **Add sample** | **10µl** |
| **Incubate** | **30minutes** |
| **Wash** | **5times** |
| **Add HRP-Conjugate** | **100µl** |
| **Incubate** | **30minutes** |
| **Wash** | **5times** |
| **Coloring** | **50µl A + 50µl B** |
| **Incubate** | **15minutes** |
| **Stop the reaction** | **50µl stop solution** |
| **Read the absorbance** | **450nm or 450/630 nm** |

## REFERENCES:


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|---|---|
| 2008-01-05 | DA-HCV-IgM-2009 |

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