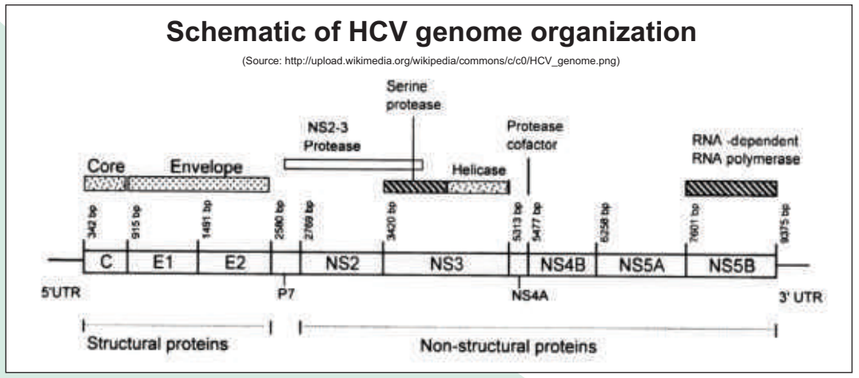
Erba Lisa® HCV Gen3 (v2)

96 tests **Product Code: 131560**

THIRD GENERATION ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF ANTIBODIES TO HEPATITIS C VIRUS IN HUMAN SERUM AND PLASMA

OVERVIEW

Hepatitis C is an infectious disease affecting the liver caused by the Hepatitis C Virus (HCV). HCV is an RNA virus now well established to be heterogeneous in nature, showing multiple genotypes and subtypes, with the basic structure and genome organization being conserved. There are six major genotypes of HCV which are indicated numerically.



With approximately 170 million people worldwide estimated to be infected with HCV, a figure four times the HIV infection status, HCV has the potential to be the next pandemic. Since the identification and molecular characterization of HCV in 1989 a variety of diagnostic tests based on the detection of anti-HCV antibodies have been developed. Hepatitis C testing begins with serological blood tests used to detect antibodies to HCV. Overall, HCV antibody tests have a strong positive predictive value for exposure to Hepatitis C.The most commonly used initial blood test for Hepatitis C is the ELISA (Enzyme-Linked Immunosorbent Assay).

INTENDED USE

Erba LISA® HCV Gen3 (v2) is an in-vitro diagnostic kit for qualitative detection of antibodies against Hepatitis C virus in patient serum / plasma. The kit is designed for use in blood banks to screen infected units as well as for clinical diagnostic laboratories.

PRINCIPLE

The **Erba LISA® HCV Gen3 (v2)** test kit is a two-step solid phase enzyme immunoassay for the qualitative detection of anti-HCV antibodies present in human serum and plasma. The kit utilizes a mixture of recombinant proteins of HCV i.e., Core, NS3, NS4 and NS5 for detection of anti-HCV antibodies. In the assay when human serum or plasma is added to the well, the bound antigen present in the well will form a stable complex with the anti-HCV antibodies present in test or positive control specimen. After washing, goat anti-human IgG (Fc)-HRPO is added to the wells. Only the bound antigen-antibody complex present in the well will react with the conjugate molecule. A second washing step will remove the unbound conjugate molecule. Addition of color reagent will develop color only in positive control wells and wells containing anti-HCV antibodies in test specimen. Upon addition of stopping solution, blue color changes to yellow. The intensity of developed yellow color is directly proportional to the presence of bound anti-HCV antibodies in the respective wells.

KIT CONTENTS

Sr. No.	Reagents/Material	Presentation
1	***Coated Microwells*** – Breakaway microwells coated with a mixture of synthetic & recombinant HCV Core, NS3, NS4 and NS5 antigens	8 wells x 12 strips
2	***Sample Diluent CV2*** – Ready to use, buffer with animal serum and detergent	12.0 mL
3	***Anti-HCV Negative Control*** – Ready to use, inactivated normal human serum	0.5 mL
4	***Anti-HCV Positive Control*** – Ready to use, human serum containing inactivated anti-HCV	0.5 mL
5	***Assay Buffer CV2*** – Ready to use use, protein-containing buffer to increase sensitivity and specificity of the conjugate	6.0 mL
6	***HCV Conjugate CV2***– Ready to use, Goat anti-human IgG (Fc) conjugated with HRPO	6.0 mL
7	***Washing Solution (20X conc.)*** – Buffer containing surfactant. Dilute 1:20 with distilled water before use	30.0 mL
8	***Color Reagent*** – Ready to use, 3,3',5,5'-Tetra methyl benzidine, Dimethyl sulfoxide, H_2O_2	6.0 mL
9	***Stopping Solution*** – Ready to use, phosphoric acid	12.0 mL
10	***Black Plate Cover*** – To avoid exposure to light during incubation	1
11	***Strip Sealers*** – Adhesive back strips for sealing strips during incubation in order to avoid evaporational loss	2
12	***Zip Lock Bag***	1

***Items 1 to 8 should be stored at 2-8°C.**

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ISO 9001, ISO 13485
QUALITY SYSTEM CERTIFIED

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MATERIALS REQUIRED BUT NOT PROVIDED

- Absorbent paper
- Disposable gloves and protective glasses
- Pipettes capable of delivering 10, 50 and 100 µL volumes
- Disposable tips
- Graduated cylinder
- Waste disposal container
- Timer
- ELISA reader (Lisa Scan™)
- Automatic / Semi-automated washing system (Lisa Wash™)
- Distilled / deionized water
- Sodium hypochlorite solution for disposal of waste
- 37°C Incubator

STORAGE

1. The shelf life of the kit is 18 months from the date of manufacturing when stored at 2-8°C.
   Do not FREEZE the components.
2. Immediately after use, return all reagents to 2-8°C.
3. The reconstituted wash buffer is stable for 2 months when stored at 2-8°C.
4. The unused microwell strips are suitable for use for 4 weeks after opening the foil pouch when stored at 2-8°C with desiccant in the Ziploc pouch.

PRECAUTIONS

- ·The test is for in-vitro diagnostic use and should be performed and interpreted by a competent or trained person only.
- DO NOT perform the test in case there is a cut or wound in the hands.
- Disposable gloves should be worn throughout the procedure.
- Before use, wait for all reagents to stabilize to room temperature.
- DO NOT use kit components beyond expiration date as indicated on the labels.
- DO NOT mix reagents between different lots as these are optimized for individual batch performance.
- All specimens and controls should be considered potentially infectious and discarded appropriately.
- Use a new tip for each controls and specimen.
- Use dedicated disposable tips to avoid microbial contamination of the reagents.
- Use clean glassware rinsed with distilled water.
- Use only distilled / deionized water to reconstitute the washing solution.
- DO NOT interchange caps of the reagents.
- Run the recommended number of positive and negative controls in each assay to ensure validity of the kit.
- Use Clear serum. Particulate matter should be removed by centrifugation.
- DO NOT allow the microplate wells to dry between the end of washing and the addition of the reagent.
- Incubation time should not vary by more than ± 2 minutes.
- After using required number of strips, rest of the strips along with activated silica gel should be kept in sealed condition into Zip lock pouch.
- DO NOT expose color reagent to light, heat, metal ions & Peroxidase.
- Level the microwell strips in the holder before reading the results. Wipe the bottom of the wells with a clean tissue to remove any dust or moisture.
- Wash hands thoroughly with a suitable detergent, after the use of kit.
- In case of an accident, such as contact with eyes or contact of contaminated material with skin wounds or ingestion of contaminated material, etc. consult a physician immediately.
- Spills should be immediately decontaminated with a suitable disinfectant.
- Prior to disposal, all waste material should be collected and soaked in 5% sodium hypochlorite for at least 30 minutes.

SPECIMEN COLLECTION AND STORAGE

Erba LISA® HCV Gen3 (v2) is recommended to be used only for testing of human serum / plasma. Collect the specimen aseptically. Extract serum as soon as possible to avoid hemolysis. Samples containing aggregates must be centrifuged prior to use.
Fresh serum / plasma samples are preferred. Undiluted serum can be stored at 2-8°C for a week or frozen at -20°C until use. Frozen specimen should be completely thawed and centrifuged. The test should be performed on clear supernatant collected after centrifugation.
Heat inactivated, hemolyzed and icteric hyperlipemic samples may yield erroneous results.

RECONSTITUTION OF REAGENTS

Dilute washing solution 1:20 in distilled or deionized water. Homogenize. Washing solution may form crystals under cold storage conditions. If so, use it after thawing at 37°C in a water bath.

ASSAY PROCEDURE

It is recommended that the assay procedure should be strictly adhered to in order to obtain reliable results.

1) Bring all the reagents and test specimens to room temperature and shake well before use.
2) Define the sample / control distribution and identification plan. In each run, assign one well for the Blank (A1), 3 wells for the Anti-HCV negative control (B1, C1, D1) and 1 well for the Anti-HCV positive control (E1).
3) Break the number of required wells for a run. Wrap the balance unused wells tightly in zip-lock pouch with desiccant and return it to 2-8°C immediately.
4) Add 100 µL of the Sample Diluent CV2 to all the wells.
   Add 10 µL of the Sample Diluent CV2 in Blank well A1.
   Add 10 µL of the Anti-HCV Negative control in wells B1, C1 and D1.
   Add 10 µL of the Anti-HCV Positive control in well E1.
   Add 10 µL of the first sample in well F1, second sample in well G1 and so on.
   Mix well, cover the wells with the strip sealers and incubate for 45 minutes at 37°C.
5) Remove the sealer. Discard / aspirate the contents of the well into the waste disposal container. Add a minimum of 350 µL of washing solution to each well. Aspirate again after 30 seconds of soak time. Repeat the washing step 5 times more (Invert the plate and tap it on absorbent pad to remove the remaining washing solution).
6) Add 50 µL of Assay Buffer CV2 & 50µL of HCV Conjugate CV2 into all the wells. Cover the wells with the strip sealers & black cover, incubate for 30 minutes at 37°C.
7) Repeat Step 5
8) Add 50µL of the color reagent to all wells. Cover the plate with the black cover provided and allow the reaction to develop in the dark for 15 minutes at room temperature (20-30°C).
9) Add 100 µL stopping solution to all wells. Homogenize. After the addition of the stopping solution the blue color of the substrate turns to yellow (for positive samples) or remains colorless (for negative samples).
10) Carefully wipe the plate bottom and Read the Optical Density at 450 nm (using 620/630/650 nm as the reference filter) within 15 mins after pipetting of stop solution. Deduct Blank absorbance from control and test wells.

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CALCULATION AND INTERPRETATION OF RESULTS

Blank value : Absorbance value (O.D.) of blank should be less than 0.1
Positive Control : Absorbance value (O.D.) of the positive control should be greater than 0.5
Negative Control : Absorbance values (O.D.) of the individual negative controls should be less than 0.1

Calculate the mean of the measured absorbance values (O.D.) for the Anti-HCV Negative Control (NCx)

Calculation of NCx:

Example:

NC	Absorbance
B1	0.021
C1	0.020
D1	0.022

NCx = (0.021+0.020+0.022) / 3 = 0.021
COV = 0.3 + 0.021 = 0.321

Calculation of the Cut-Off Value (COV)

COV = 0.3 + NCx

Interpretation of the result:
Non-Reactive: Samples with an optical density less than the cut-off value are considered Non-Reactive.

Reactive: Samples with an optical density equal to or greater than the cut-off value are considered Initial Reactive.
These samples should be retested in duplicate. On retesting if the optical density of the duplicates is less than the cut-off value, the specimen is considered as Non-Reactive. If the retest result of the duplicates is found reactive, the specimen is considered Repeatedly Reactive.

Repeatedly Reactive specimens identified using **Erba LISA® HCV Gen3 (v2)** , must be further confirmed using other tests such as RIBA or qualitative PCR for HCV RNA detection.

PERFORMANCE CHARACTERISTICS

The performance of ERBA LISA® HCV Gen3 (v2) has been determined in house using a panel containing 164 Anti-HCV positive samples and 705 anti-HCV negative samples.

The results of the in house study are as follows:

Sample Description	Erba LISA® HCV Gen3 (v2)	
	Reactive	Non-Reactive
Anti-HCV Positive	164	0
Anti-HCV Negative	0	705

Sensitivity – 100% **Specificity – 100%**

Note:

1. Using **Erba Lisa HCV Gen3 (v2)** Kits, in some instances, it is possible to obtain OD values of controls and samples preceded by a negative sign. An OD up to -0.05 is acceptable and can be considered Zero OD for calculation of the cut-off value. This will, in no way, affect the assay performance or the test results.

2. The ELISA readers have a linear measuring range approximately 2.5A. Beyond this range OD values are non-linear. Therefore many instruments programmed to show 'OUT' or 'OUT OF RANGE' indication, if the OD exceeds 2.5 or 3 or more, even after dilution of sample or control. Please note that this indicates a valid run provided the negative control value is <0.1 and does not interfere with assay results.

LIMITATIONS OF THE TEST

1. **Erba LISA® HCV Gen3 (v2)** is a screening test. All reactive samples should be further confirmed by supplemental assays such as RIBA, qualitative PCR etc.
2. A non-reactive test does not exclude the possibility of an HCV infection.
3. **Erba LISA® HCV Gen3 (v2)** detects only the IgG type of anti-HCV.
4. False positive results may occur due to non-specific binding of either the sample or the conjugate to the microwell.
5. The assay is only valid for human serum and plasma samples and not for other body fluids.
6. In case the kit is not stored properly or the test is not performed as per the recommended instructions, it may lead to erroneous results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

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REFERENCES

1. Dienstag J. L.(1983). Non-A, Non-B Hepatitis, I. Recognition, epidemiology and clinical features. Gastroenterology, 85, 439-462.
2. Alter H. J.(1988). Transfusion associated non-A, non-B Hepatitis: the first decade, p537-542. In Viral Hepatitis and Liver disease, Ed. A. J. Zukerman, Alan R. Liss, Inc., New York.
3. Choo O. L. et al.(1989). Isolation cDNA clone derived from a blood-borne, non-A, non-B viral hepatitis genome. Science, 224, 359-362.
4. Dawson G. J. et al (1991). Detection of antibodies to Hepatitis C Virus in US Blood Donors. J. Clin. Microbiol, 551-556.
5. Kuog G., et al(1989). An assay for circulating antibodies to a major aetiologic virus of human non-A, non-B hepatitis. Science, 244, 363-364.
6. Uytrendaele S., et al(1994). Evaluation of third generation screening and confirmatory assays for HCV antibodies. Vox Sang, 66, 122-129.

For IVD Use Only

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