fortress diagnostics

ISO 13485 accredited company

BXE0783A

96 TESTS

STORE AT 2-8ºC

FOR IN-VITRO DIAGNOSTICS USE ONLY

Anti HCV ELISA (HS)

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of anti-HCV ELISA achieved.

INTENDED USE

anti-HCV ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to hepatitis C virus (HCV) in human serum or plasma. It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis C virus.

SUMMARY

Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form although more than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced.

- The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens.
- Second generation tests, which included recombinant / synthetic antigens from the Core (c22) and non-structural regions NS3 (c33c, c100-3) and NS4 (c100 3, c200) resulted in a remarked improvement in sensitivity and specificity.
- The third generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Third generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies (window period) to 60 days.

anti-HCV ELISA is based on double antigen "sandwich" principle ELISA. This novel for the testing of HCV antibodies method allows detection of very early antibodies including IgM, and IgA in addition to the IgG which is the main target for detection of the previous generation assays. In addition, the method minimizes the unspecific reaction showed by the other methods and thus its utilization increases the specificity in detection.

PRINCIPLE OF THE TEST

This kit is a two-step incubation enzyme immunoassay, which uses polystyrene microwell strips pre-coated with recombinant HCV antigens expressed in E.coli (recombinant Core and NS3/4/5). Patient's serum or plasma sample is added together with biotin-conjugated HCV antigens. During the first incubation step, the specific HCV antibodies, if present, will be captured inside the wells as a double antigen "sandwich" complex comprising of the coated, and the biotin-conjugated HCV antigens. The microwells are then washed to remove unbound serum proteins. During the second incubation step, the captured HCV antibodies are detected by adding of HRP-Conjugate. The microwells are then washed to remove unbound conjugate, and Chromogen solutions are added to the wells. In wells positive for HCV antibodies, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibodies captured in the wells, and to the sample respectively. Wells containing samples negative for anti HCV remain colorless.

COMPONENTS

IVD **In Vitro Diagnostic Use Only**

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

Code / Size	Component / Description
CODE 5 (1x96 wells) 8×12/12×8 well per plate	**MICROWELL PLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HCV antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once open, stable for one month at 2-8°C.
CODE 8 (1x1ml per vial) preserv.0.1% ProClinTM 300	**NEGATIVE CONTROL:** Blue-colored liquid filled in a vial with green screw cap. Protein-stabilized buffer tested non-reactive for HCV antibodies. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 7 (1x1ml per vial) preserv.0.1% ProClinTM 300	**POSITIVE CONTROL:** Red-colored liquid filled in a vial with red screw cap. HCV antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 6 (1x12ml per vial) preserv.0.1% ProClinTM 300	**HRP-CONJUGATE:** Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated avidin. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 10 (1x6ml per vial) preserv.0.1% ProClinTM 300	**BIOTIN CONJUGATE:** Blue-colored liquid in a vial with blue screw cap. Biotinylated HCV antigens diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8 C.
CODE 1 (1x50ml per bottle) ***DILUTE BEFORE USE!*** detergent Tween-20	**WASH BUFFER:** Colorless liquid filled in a clear bottle with white screw cap. PH 7.4, 20 × PBS 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. when stored at 2-8°C.
CODE 2 (1x6ml per vial)	**CHROMOGEN SOLUTION A:** Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 3 (1x6ml per vial)	**CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with black screw cap.TMB solution (Tetramethyl benzidine). Ready to use as supplied. Once open, stable for one month at 2-8°C.
CODE 4 (1x6ml per vial)	**STOP SOLUTION:** Colorless liquid in a white vial with white screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once open, stable for one month at 2-8°C.

PLASTIC SEALABLE BAG: For enclosing the strips not in use 1 unit
PACKAGE INSERT: 1 copy
CARDBOARD PLATE COVER: 3 sheets
To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±0.5°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTING AND STORAGE

1. Specimen Collection: No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with 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 haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin may be tested, but **highly lipaemic, icteric, or hemolytic specimens should not be used** as they can give false results in the assay. **Do not heat inactivate specimens.** This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. **anti-HCV ELISA is intended ONLY** for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimen 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 transportation of clinical samples and ethological agents.

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 anti HCV ELISA , during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS
The ELISA assays are time and temperature sensitive. 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 for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C 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. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. 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.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, 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.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, 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. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT:

Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators 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 and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Fortress technical support for further assistance.

ProClin™ 300
S phrases:
S26-28-36/37/39-45-60-61
R phrases: 43

Do not eat and drink at the laboratory

Ware protective clothing
Ware eye protection

Biohazard
Danger

Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom)
Tel: +44 (0) 2894 487676 | Fax: +44 (0) 2894 469933 | Website: www.fortressdiagnostics.com

CE 1293

PROCEDURE

Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash buffer (20X) as indicated in the washing step instructions. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

STEP 1	Preparation: Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP Conjugate, or BIOTIN-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.
STEP 2	Adding BIOTIN-Conjugate Reagent: Add 50µl of BIOTIN-Conjugate Reagent into each well except the Blank.
STEP 3	Adding Sample: Add 50µl of Positive control, Negative control, and Specimen into their respective wells except the Blank. Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination. Mix by tapping the plate gently.
STEP 4	Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.
STEP 5	Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
STEP 6	Adding HRP-Conjugate: Add 100µl HRP-Conjugate into each well except the Blank, and mix by tapping the plate gently.
STEP 7	Incubating: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
STEP 8	Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
STEP 9	**Coloring:** Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 30 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HCV antibody positive sample wells.
STEP 10	Stopping Reaction: Using a multichannel pipette or manually, add 50µl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and HCV antibody positive sample wells.
STEP 11	Measuring theAbsorbance: 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 10 minutes after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order 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 of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer 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 carry out 5 washing cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. 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 at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) 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 A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = Nc + 0.12 (Nc = the mean absorbance value for three negative controls).

Quality control (assay validation): The test results are valid if the Quality Control criteria are fulfilled. 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.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

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

Example:
1. Quality Control
Blank well A value: A1= 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range
2. Calculation of Nc: = (0.020+0.012+0.016) = 0.016
3
3. Calculation of the Cut-off: (C.O.) = 0.016 +0.12 = 0.136

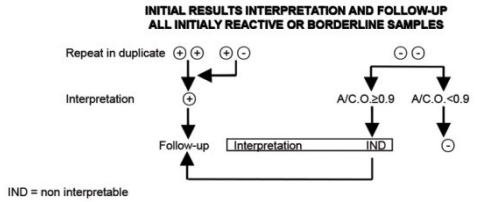
INTERPRETATIONS OF THE RESULTS

Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis C virus antibodies have been detected with anti-HCV ELISA , therefore the patient is probably not infected with HCV and the blood unit do not contain HCV antibodies and could be transfused in case that other infectious diseases markers are also absent.

Positive Results (A / C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that hepatitis C virus antibodies have probably been detected using anti-HCV ELISA . All initially reactive specimens should be retested in duplicates using anti-HCV ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for hepatitis C virus antibodies with anti-HCV ELISA.

Borderline (A / C.O. = 0.9-1.1): Specimens with absorbance to Cut off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. RIBA, PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.



IND = non interpretable

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding Fortress ELISATroubleshooting, please refer to Fortress's "ELISAs and Troubleshooting Guide".
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for hepatitis C virus antibodies and therefore the patient is probably infected with HCV and the blood unit must be discarded.
- After retesting in duplicates, samples with values close to the Cut off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

Evaluation studies carried out in Paul-Ehrlich-Institut (PEI), German Red Cross Institute Baden-Württemberg – Hessen, Sanquin Bloedvoorziening, and six blood banks and hospitals in China, demonstrated the following performance characteristics of anti-HCV ELISA . Diagnostic specificity: When evaluated on European blood donors (n=5083), the overall diagnostic specificity of the kit was 99.96%. On Chinese blood donors (n=15,997), the overall diagnostic specificity of the kit was 99.97%.

Analytical specificity:

Testing Laboratory : German Red Cross - Baden-Württemberg Hessen, Germany. Total of 210 blood samples from hospitalized patients were evaluated. All samples selected were found negative on the reference test. Among these patients, six samples were initially reactive. One of them remained positive after repeat testing in duplicate. None of them showed anti-HCV antibodies with the confirmation test on immunoblot (Innogenetics INNO-LIA HCV). 100 samples with potentially cross reacting substances were evaluated. None of them were reactive with the anti-HCV ELISA .

No interference was observed with samples from patients with high-level of rheumatoid factor, and pregnant woman.
Same day and frozen specimens have been tested to check for interferences due to collection and storage.

Sensitivity:

Testing Laboratory: Paul-Ehrlich Institut (PEI-IVD), Germany. In total 397 anti-HCV positive serum or plasma samples were tested, encompassing 200 genotypes samples, representing the major HCV genotypes 1-6. With respect to diagnostic sensitivity on clinical specimens and HCV genotypes 1-6, anti-HCV ELISA detected all positive samples as positive.

anti-HCV ELISA was also evaluated for sensitivity on 33 HCV commercial available seroconversion panels. The seroconversion sensitivity of the assay was comparable to other CE-marked HCV antibody screening assays.

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease. Therefore, negative results obtained with anti-HCV ELISA are only indication that the sample does not contain detectable level of hepatitis C virus antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HCV or the blood unit is not infected with HCV.
3. 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. For more information regarding Fortress ELISA Troubleshooting, please refer to Fortress's "ELISAs and Troubleshooting Guide", or contact Fortress technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) plasma. anti-HCV ELISA has been evaluated only with individual serum or plasma specimens.
7. anti-HCV ELISA is a qualitative assay and the results cannot be use to measure antibodies concentration.

REFERENCES

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2. Alter HJ., Purcell RH, Holland PV, et al. (1978) Transmissible agent in non-A, non-B hepatitis. Lancet I: 459-463.
3. Choo Q-L,Weiner AJ, Overby LR, Kuo G, Houghton M. (1990) Hepatitis C Virus: the major causative agent of viral non-A, non-B hepatitis. Br Med Bull 46: 423-441.
4. Engvall E, Perlmann P. (1971) Enzyme linked immunosorbent assay (ELISA): qualitative assay of IgG. Immunochemistry 8:871-874.

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1	Microwell plate	Code 5	one
2	Negative Control	Code 8	1x1ml
3	Positive Control	Code 7	1x1ml
4	HRP-Conjugate	Code 6	1x12ml
5	BIOTIN-Conjugate	Code 10	1x6ml
6	Wash Buffer	Code 1	1x50ml
7	Chromogen Solution A	Code 2	1x6ml
8	Chromogen Solution B	Code 3	1x6ml
9	Stop Solution	Code 4	1x6ml

SUMMARY OF THE ASSAY PROCEDURE:

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add BIOTIN-Conjugate	50µl
Add Samples	50µl
Incubate	60 minutes
Wash	5 times
Add HPR-Conjugate	100µl

Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	30 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/630 nm

EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	...										
C	Neg.	...										
D	Neg.											
E	Pos.											
F	Pos.											
G	S1											
H	S2											

CE MARKING SYMBOLS:

IVD For In Vitro Diagnostics Use Only

LOT Batch

REF Catalogue Number

Storage Temperature Conditions

Use By / Expiry Date (Year / Month)

Warning, Read Enclosed Documents

Instructions For Use

Manufactured By

Fortress Diagnostics Limited,
Unit 2C Antrim Technology Park,
Antrim, BT41 1QS (United Kingdom)
Tel: +44 (0) 2894 487676
Fax: +44 (0) 2894 469933
Website: www.fortressdiagnostics.com

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