AccuDiag™ Syphilis IgG/IgM ELISA Kit

REF 1465-12

IVD 8°C 2°C Σ 96 Tests

Test	Syphilis IgG/IgM (anti-TP)
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA: Antibody coated plate
Detection Range	Qualitative: Positive, Negative Control & Cut Off
Sample	20 µL
Total Time	~75 min
Shelf Life	12 Months
Specificity	99.95 %
Sensitivity	99.52%

*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 DAI Syphilis IgG/IgM ELISA achieved.

INTENDED USE

The Diagnostic Automation, Inc. (DAI) **Syphilis IgG/IgM ELISA** is an enzyme-linked immunosorbent assay for the qualitative detection of antibodies to Treponema palladium (TP) in human serum/plasma. The Syphilis IgG/IgM ELISA test is intended for screening of blood donors and as an aid in the diagnosis of clinical conditions related to infection with Treponema Pallidum.

SUMMARY

Syphilis is a disease caused by Spirochete bacterium called Treponema Pallidum (TP). If untreated, the organisms move throughout the body and can cause damage to many organs, making syphilis a life-threatening disease if not treated early enough. People who have been infected with Syphilis experience different symptoms during the 3 stages of the disease. Early, which is defined by the presence of the chancre at the site of inoculation. Syphilis may be further divided into primary, secondary, and early latent syphilis; late syphilis includes late latent and the various forms of tertiary Syphilis. The serological response to syphilis involves production of antibodies to a wide range of antigens, including non-specific antibodies and specific anti-TP antibodies. The first detectable response to infection is the production of specific antitreponemal IgM, which can be detected within 4 to 7 days after the chancre appears and until the end of the second week of infection; antitreponemal IgG appears at about four weeks later. By the time that symptoms develop, most patients have detectable IgG and IgM.

TEST PRINCIPLE

With DAI Syphilis ELISA, the detection of anti-TP antibodies is achieved by antigen ―sandwich‖ enzyme-linked method (ELISA) where polystyrene microwell strips are pre-coated with recombinant Treponema Pallidum antigens expressed in E. coli. The sample is incubated in the microwells together with recombinant TP antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-TP in the sample, during incubation the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and the specific antigens-antibody immunocomplex is captured on the solid phase. After washing to remove sample and unbound conjugates, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added into the wells. In presence of the antigen-antibody-antigen ―sandwich‖ complex, 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 can be measured and is proportional to the amount of antibody in the sample. Wells containing samples negative for anti-TP remain colorless.

MATERIALS AND COMPONENTS

Materials provided with the test kits

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

MICROWELL PLATE 1plate
Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant.
12×8-well strips per plate. Each well contains recombinant TP antigens.
The microwell strips can be broken to be used separately.
Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return at 2~8°C.

NEGATIVE CONTROL 1vial
Yellowish liquid filled in a vial with green screw cap.
0.5ml per vial.
Protein-stabilized buffer tested non-reactive for TP.
Preservatives: 0.1% ProClin 300™.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

POSITIVE CONTROL 1vial
Red-colored color liquid filled in a vial with red screw cap.
0.5ml per vial.
Antibodies to TP diluted in protein-stabilized buffer.
Preservatives: 0.1% ProClin 300™.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

HRP-CONJUGATE REAGENT 1vial
Green-colored liquid filled in a white vial with red/orange screw cap.
14ml per vial.
Horseradish peroxidase-conjugated TP antigens.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

WASH BUFFER 1bottle
Colorless liquid filled in a clear bottle with white screw cap.
50ml per bottle.
PH 7.4 20 × PBS. (Containing Tween-20 as a detergent).
DILUTE BEFORE USE -The concentration 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°C.

CHROMOGEN SOLUTION A 1vial
Colorless liquid filled in a white vial with green screw cap.
8ml per vial.
Urea peroxide solution.
Ready to use as supplied.
Once open, stable for 4 weeks at 2-8°C.

CHROMOGEN SOLUTION B 1vial

Colorless liquid filled in a black vial with black screw cap.
8ml per vial
TMB solution (Tetramethyl benzidine) solution.
Ready to use as supplied.
Once open, stable for 4 weeks at 2-8°C.

STOP SOLUTION 1vial
Colorless liquid filled in a white vial with yellow screw cap.
8ml per vial Diluted sulfuric acid solution (0.5M H_2SO_4).
Ready to use as supplied.
Once open, stable for 4 weeks at 2-8°C.

PLASTIC SEALABLE BAG 1unit
For enclosing the strips not in use.

CARDBOARD PLATE COVER 2sheets
To cover the plates during incubation and prevent evaporation or contamination of the wells.

PACKAGE INSERTS 1copy

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±1°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, microwell aspiration/wash system.

SPECIMEN COLLECTION AND PREPARATION

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 can 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. The DAI Syphilis IgG/IgM 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. Specimens not required for assaying within 1 week 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.

PRECAUTIONS

This kit is intended **FOR PROFESSIONALS 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 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.
19. Materials Safety Data Sheet (MSDS) available upon request.
20. 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.
21. The Stop solution 0.5M H2SO4 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.
22. ProClin™ 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 Diagnostic Automation, Inc. technical support for further assistance.

SPECIAL 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.

ASSAY PROCEDURE

Reagents preparation: Allow the reagents and samples 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, re-solubilize 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 buffer.

Step1 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 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.

Step2 Adding HRP-Conjugate: Add **100µl** HRP-Conjugate into each well except the Blank.

Step3 Adding Sample: Add **20µl** of Positive control, Negative control, and specimen into their respective wells - the HRP-Conjugate-sample mixture in the wells will change the color from **GREEN** to **BLUE** after adding of the samples. **Note: Use a separate disposal pipette tip for each specimen, Negative , Positive Control to avoid cross-contamination. Mix by tapping the plate gently.**

Step4 Incubating: Cover the plate with the platecover and incubate for **60minutes at 37°C**

Step5 Washing: At 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 down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step6 Coloring: Add 50µl of Chromogen Solution A and 50µl of Chromogen Solution B into each well including the Blank. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and anti-TP positive sample wells.

Step7 Stopping Reaction: Using a multichannel pipette or manually, add 50µl of Stop Solution into eachwell and mix gently. Intensive yellow color develops in Positive control and anti-TP positive sample wells.

Step8 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)

RESULTS AND QUALITY CONTROL

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.

1. Calculation of Cut-off value (C.O.) = *Nc + 0.18
***Nc = the mean absorbance value for three negative controls**

2. Quality control range:
The test results are valid if the Quality Control criteria are verified. It is advisable 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.

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.

Example:
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

Calculation of Nc: = (0.020+0.012+0.016)/3 = 0.016

Calculation of the Cut-off: (C.O.) = 0.016 +0.18 = 0.196

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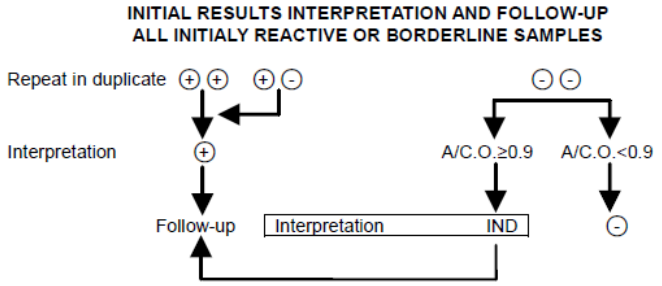
3. Interpretations of the results:
Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-TP antibodies have been detected with DAI Syphilis IgG/IgM ELISA, therefore there are no serological indications for current infection with TP.

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 anti-TP antibodies have probably been detected using DAI Syphilis IgG/IgM ELISA. All initially reactive specimens should be retested in duplicates using DAI Syphilis IgG/IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to TP with DAI Syphilis IgG/IgM 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 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 DAI's ELISA Troubleshooting, please refer to DAI's ELISAs and Troubleshooting Guidel.
- 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 antibodies to TP and therefore the patient is probably infected with TP.
- 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

The clinical performances of this assay have been evaluated by a panel of samples obtained from 4293 healthy blood donors from 8 blood banks. The sensitivity of DAI Syphilis IgG/IgM ELISA of blood donors were 99.52% (413/415), while the specificity were 99.95% (3859/3861).

Testing Center	TPPA Positive	TPPA Suspicious	TPPA Negative
Blood Bank 1	14/14	-	2888/2890
Blood Bank 2	6/6	-	521/521
Blood Bank 3	76/76	4/6	59/59
Blood Bank 4	8/8	0/2	148/148
Blood Bank 5	90/90	1/1	13/13
Blood Bank 6	80/80	-	12/12
Blood Bank 7	2/2	0/2	148/148
Blood Bank 8	137/139	5/6	70/70
Total	413/415	-	3859/3861

The serum samples collected from 222 syphilitic patients who had been diagnosed correctly by clinic (including 1st period, 2nd period, 3rd period and latent period Syphilis), 42 autoimmune diseases patients excluding syphilis (RPR testing positive) and 270 healthy blood-donors, then they were tested for antibody against treponema pallidum by RPR, TPPA and TP-ELISA methods respectively. Results: The positive rates of TP-ELISA, TPPA and RPR for detection of antibody against treponema pailidum were 97.30% (216/222), 95.95% (213/222) and 90.54% (201/222) respectively. There was no statistical difference between TPPA and TP-ELISA for diagnose of syphilis (P>0.05). It didn't appear false positive in TPPA and TP-ELISA methods to 42 autoimmune diseases patients excluding syphilis

Samples		TP ELISA		TRUST/RPR		TPPA/TPHA	
Syphilis	No	+	-	+	-	+	-
1st period	66	60	6	55	11	59	7
2nd period	140	140	0	131	9	138	2
3rd period	2	2	0	2	0	2	0
latent period	14	14	0	13	1	14	0
TOTAL	222	216	6	201	21	213	9

Analytical Specificity:
No cross reactivity was observed with specimens from patients infected with HAV, HCV, HBV, HTLV, CMV, and HIV.
No interference was observed from rheumatoid factors up to 2000U/ml.
No high dose hook effect observed during clinical testing.
The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.

LIMITATIONS OF PROCEDURE

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 and in some immunosuppressed individuals. Therefore, negative results obtained with DAI Syphilis IgG/IgM ELISA are only indication that the sample does not contain detectable level of anti-TP antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with TP.
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 DAI's ELISA Troubleshooting, please refer to DAI's ELISAs and Troubleshooting Guidel, or contact DAI 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) serum or plasma. DAI Syphilis IgG/IgM ELISA has been evaluated only with individual serum or plasma specimens.
7. DAI Syphilis IgG/IgM ELISA is a qualitative assay and the results cannot be used to measure antibody concentration.

REFERENCE

1. Fraser CM, et al. Complete genome sequence of Treponema pallidum, the syphilis spirochete. Science 1998; 281:375.

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

2. Holmes KK, Lemon SM, Mardh P, Piot P, Sparling PF, Stamm WE, Wasserheit JM, Weisner PF. Chapters 33-36. In Sexually transmitted diseases, 3rd ed. New York: McGraw-Hill, 1999.
3. Hook EW III, Martin DH, Stephens J, Smith BS, Smith K. A randomized, comparative pilot study of azithromycin versus benzathine penicillin G for treatment of early syphilis. Sex Transm Dis 2002 Aug; 29(8):486-490.
4. Hook EW III, Stephens J, Ennis DM. Azithromycin compared with penicillin G benzathine for treatment of incubating syphilis. Ann Intern Med 1999 Sept 21; 131(6):434-437.
5. Johns DR, Tierney M, Felsenstein D. Alteration in the natural history of neurosyphilis by concurrent infection with the human immunodeficiency virus. N Engl J Med 1987; 316:1569-72.

ISO 13485
ISO 9001

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, California 91367 USA

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REF 1465-12	AccuDiag™ - Syphilis IgG/IgM ELISA

Revision Date: 2014-01

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	one
2. Negative Control	1x0.5ml
3. Positive Control	1x0.5ml
4. HRP-Conjugate	1x14ml
5. Wash Buffer	1x50ml
6. Chromogen Solution A	1x8ml
7. Chromogen Solution B	1x8ml
8. Stop Solution	1x8ml

SUMMARY OF THE ASSAY PROCEDURE

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

Add HPR-Conjugate	100µl
Add Samples / Controls	20µl
Incubate	60minutes
Wash	5times
Coloring	50µl A + 50µl B
Incubate	15minutes
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											