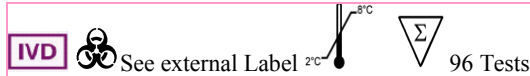


AccuDiag™
Syphilis (TPA) IgM
ELISA Kit

REF 1464-6



Test	Syphilis ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative: Positive & Negative Control
Sample	10 µL serum
Total Time	~ 95 min
Shelf Life	12 months from the manufacturing date
Specificity	95 %
Sensitivity	95 %

INTENDED USE

The **DAI Syphilis (TPA) IgM Enzyme Immunoassay Kit** provides materials for the **qualitative** and **semiquantitative** determination of IgM-class antibodies to *Treponema pallidum* in human serum and plasma.

This assay is intended for in vitro diagnostic use only.

SUMMARY AND EXPLANATION

Spirochetes are motile bacteria with a periplasmic axial filament. All pathogenic species belong to the family Treponemataceae, which includes the three genera: *Treponema*, *Borrelia*, and *Leptospira*. The *Treponema* are motile bacteria, 5-15µ in length and 0.2µ in width, containing about 10 flexible, undulating, spiral shaped rods. *Treponema pallidum*, the causative agent of Syphilis, is transmitted by direct contact, usually through sexual intercourse. Syphilis along with Gonorrhoea, Chancroid and Lymphogranuloma venereum, designated as a venereal disease, or VD, is an acute and chronic infectious disease. After an incubation period of 12-30 days, the first symptoms to appear are chancres, soon followed by syphilitic ulcers which then spontaneously disappear in a few weeks. During this first stage (primary syphilis) the *Treponema pallidum* propagates in related lymph nodes to be distributed to the whole body stream. Three further stages of disease follow which are classified as secondary, tertiary, and quaternary syphilis. Treatment with antibiotics at the earliest disease stage and prophylactic measures are ways to prevent epidemics. For this purpose, antenatal and donor blood screenings are mandatory in most of countries around the world.

TEST PRINCIPLE

The **DAI Syphilis (TPA) IgM ELISA Kit** is a solid phase enzyme-linked immunosorbent assay (ELISA). Patient samples are diluted with *Sample Diluent* and additionally incubated with *IgG-RF-Sorbent*, containing hyperimmune anti-human IgG-class antibody to eliminate

competitive inhibition from specific IgG and to remove rheumatoid factors. This pretreatment avoids false negative or false positive results.

Microtiter wells as a solid phase are coated with *Treponema pallidum* antigen.

Pretreated patient specimens and **ready-for-use controls** are pipetted into these wells. During incubation *Treponema pallidum*-specific antibodies of positive specimens and controls are bound to the immobilized antigens.

After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.

The intensity of this color is directly proportional to the amount of *Treponema pallidum*-specific IgM antibody in the patient specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay. DO not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 3 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

Prior to assaying each patient specimen is first to be diluted with *Sample Diluent*. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with *IgG-RF-Sorbent*

1. Dilute each patient specimen **1+50** with *Sample Diluent*; e.g. 10 µL of specimen + 0.5 mL of *Sample Diluent*. **Mix well.**
2. Mix well the *IgG-RF-Sorbent* before use.
3. Dilute this prediluted sample **1+1** with *IgG-RF-Sorbent* e.g. 60 µL prediluted sample + 60 µL *IgG-RF-Sorbent*. **Mix well.**
4. **Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.**
5. Take 100 µL of these pretreated samples for the ELISA.

Please note: Controls are ready for use and must not be diluted!

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Microtiterwells:** 12 x 8 (break apart) strips, 96 wells; Wells coated with *Treponema pallidum* antigen. (incl. 1 strip holder and 1 cover foil).
2. **Sample Diluent:** 1 vial, 100 mL, ready to use, colored yellow; pH 7.2 ± 0.2.
3. **IgG-RF-Sorbent:** 1 vial, 6.5 mL, ready to use, colored yellow; Contains anti-human IgG-class antibody.
4. **Pos. Control:** 1 vial, 2.0 mL, ready to use; colored yellow, red cap.



5. **Neg. Control:** 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
6. **Cut-off Control:** 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
7. **Enzyme Conjugate:** 1 vial, 20 mL, ready to use, colored red, antibody to human IgM conjugated to horseradish peroxidase.
8. **Substrate Solution:** 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
9. **Stop Solution:** 1 vial, 14 mL, ready to use, contains 0.2 mol/l H₂SO₄, Avoid contact with the stop solution. It may cause skin irritations and burns.
10. **Wash Solution:** 1 vial, 30 mL (20X concentrated for 600 mL), pH 6.5 ± 0.1 see "Preparation of Reagents".

* Contains non-mercury preservatives.

Materials required but not provided

1. A microtiter plate calibrated reader (450/620nm ±10 nm) DAR 800.
2. Calibrated variable precision micropipettes.
3. Incubator 37 °C.
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Deionized or (freshly) distilled water
7. Timer
8. Absorbent paper

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution 1+19 (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2.

Consumption: ~ 5 mL per determination.

Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use.

The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, DAI has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

PRECAUTIONS

- This kit is for in vitro diagnostic use only. For professional use only.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Avoid contact with Stop Solution containing 0.2 mol/L H₂SO₄. It may cause skin irritation and burns.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.

- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
- Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets.
- Safety Data Sheets for this product are available upon request directly from DACD.

ASSAY PROCEDURE

General Remarks

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37°C incubation cover microtiter strips with foil to avoid evaporation.

TEST PROCEDURE



Prior to commencing the assay, dilute *Wash Solution*, **prepare patient samples as described in point 5.3** and establish carefully the **distribution and identification plan** supplied in the kit for all specimens and controls.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable **calculate the mean absorbance values** of all duplicates.

1. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well (e.g. A1) for the substrate blank,

1 well (e.g. B1) for the *Neg. Control*,

2 wells (e.g. C1+D1) for the *Cut-off Control* and

1 well (e.g. E1) for the *Pos. Control*.

It is left to the user to determine controls and patient samples in duplicate.

2. Dispense

100 µL of *Neg. Control* into well B1

100 µL of *Cut-off Control* into wells C1 and D1

100 µL of *Pos. Control* into well E1 and

100 µL of each *pre-treated* sample with new disposable tips into appropriate wells.

Leave well A1 for substrate blank!

3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C**.

4. Briskly shake out the contents of the wells.

Rinse the wells **5 times** with diluted *Wash Solution* (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note:

The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense **100 µL** *Enzyme Conjugate* into each well, **except A1**.

6. Incubate for **30 minutes at room temperature (20 °C to 25 °C)**.

Do not expose to direct sun light!

7. Briskly shake out the contents of the wells.

Rinse the wells **5 times** with diluted *Wash Solution* (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets

8. Add **100 µL** of *Substrate Solution* into all wells.

9. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.

10. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.

Any blue color developed during the incubation turns into yellow.

Note: Highly positive patient samples can cause dark precipitates of the chromogen!

11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the *Stop Solution*.

Measurement

Adjust the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

RESULTS

Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

Substrate blank in A1: Absorbance value **lower than 0.100**

Neg. Control in B1: Absorbance value **lower than 0.200**

Cut-off Control in C1/D1: Absorbance value **between 0.350 - 0.800**

Pos. Control in E1: Absorbance value **between 0.650 - 3.000**

Calculation

Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).

Example: $(0.44 + 0.46) / 2 = 0.45 = CO$

Interpretation

POSITIVE Patient (mean) absorbance values more than 10 % above CO (Mean OD patient > 1.1 x CO)

GREY ZONE Patient (mean) absorbance values from 10 % above to 10 % below CO

repeat test 2 - 4 weeks later - with new patient samples
($0.9 \times CO \leq \text{Mean OD patient} \leq 1.1 \times CO$)

Results in the second test again in the grey zone \Rightarrow **NEGATIVE**

NEGATIVE Patient (mean) absorbance values more than 10 % below CO (Mean OD patient < 0.9 x CO)

Results in DAI Units [DU]

Patient (mean) absorbance value x 10 = [DAI Units = DU]
CO

Example: $\frac{1.580 \times 10}{0.45} = 35 \text{ DU}$

Interpretation of Results

Cut-off value: 10 DU

Grey zone: 9 - 11 DU

Negative: < 9 DU

Positive: > 11 DU

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.52 - 60 DU/mL.

Specificity of Antigen (Cross Reactivity)

The antigen used for the DAI Syphilis (TPA) IgM ELISA shows no cross-reactivity to Epstein Barr Virus (VCA), Mycoplasma pneumonia, and Borrelia burgdorferi IgM antibodies.

Analytical Sensitivity

The analytical sensitivity of the DAI ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.52 DU/mL (OD450 = 0.025).

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of DAI ELISA. 77 samples, therefrom 57 negative samples are assayed with DAI ELISA lot 1-3.)
It is 100% (for all three lots).

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. (Detected by method comparison with Mikrogen ELISA, with three lots of DAI ELISA. 77 samples, therefrom 20 positive samples are assayed with DAI lot 1-3.) It is 100% (for all three lots).

Method Comparison

The DAI Syphilis (TPA) IgM ELISA was compared with another Treponema pallidum IgM ELISA (Mikrogen). 77 serum samples are assayed.

n= 77		Other ELISA	
		pos.	neg.
DAI ELISA Lot 1	pos.	20	0
	neg.	0	57

Agreement: 100%

Reproducibility

Intra-assay

The intra-assay (within-run) precision of the DAI Syphilis (TPA) IgM ELISA was determined by 20 x measurements of 12 serum samples covering the whole measuring range.

Sample	Mean OD ₄₅₀	Intra-Assay CV (%)	n
1	0,37	8,30	20
2	0,24	9,64	20
3	0,51	8,65	20
4	0,94	6,29	20
5	0,94	7,11	20
6	0,67	6,51	20
7	1,30	3,72	20
8	1,35	3,42	20
9	1,44	3,35	20
10	1,96	2,48	20
11	2,08	2,85	20
12	1,62	4,75	20

Inter-assay

The inter-assay variation of the DAI Syphilis (TPA) IgM ELISA was determined with 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean OD ₄₅₀	Inter-Assay CV (%)	n
1	1,86	2,75	40
2	1,20	3,31	40
3	1,44	2,69	40

Linearity

Three samples (serum) containing different amounts of analyte were serially diluted with sample diluent and assayed with the DAI ELISA. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

		Serum 1	Serum 2	Serum 3
Concentration	DU/mL	42,66	34,23	44,58
Average % Recovery		97,81	104,93	92,09
Min Recovery	from	86,59	95,07	85,98
Max Recovery	to	112,41	114,17	97,53
Status Linearity (100 +/-15%)		passed	passed	passed

QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DAI directly.

LIMITATIONS OF PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. In immunocompromised patients and newborns serological data only have restricted value.

Interfering Substances

In general, haemolytic, icteric or lipaemic samples should be avoided, but can be tolerated up to at least 4 mg/mL haemoglobin, 0.5 mg/mL Bilirubin, and 30 mg/mL triglycerides.

None of the following samples with interference factors will interfere with the ELISA: samples with rheumatoid factor, samples with pregnancy hormones, samples with tumor marker (CYFRA, CA-72-4, CA-21-1, CA-15-3), samples with HAMA, samples with ANA and samples from elderly with high amount of proteins.

LEGAL ASPECTS

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DAI.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated.

Any laboratory result is only a part of the total clinical picture of a patient. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results are also invalid.

Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.



STORAGE

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

REFERENCE

1. Penn. C. W., M. J. Baily, and Cockayne. 1985. The axial filament antigen of *Treponema pallidum*. *Immunology* 54: 635-641
2. Luger, A., B.L. Schmidt, und F. Gschnait. 1983. Neue Fortschritte der Syphilisserologie. *Wr. Klein. Wsch.* 95: 440- 443

<p>ISO 13485 ISO 9001</p>  <p> Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street , Suite 102 and 116, Woodland Hills, California 91367 USA</p>	
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