AccuDiag™ dsDNA ELISA Kit

REF 2553-P1

IVD See External Label 2°C 8°C 96 Tests

dsDNA ELISA	
Principle	Indirect ELISA
Detection	Semi-Quantitative
Sample	10 µL serum/plasma
Incubation Time	65 minutes
Sensitivity	100%
Specificity	97.9%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI dsDNA Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection of antibodies in human serum to dsDNA antigen and as an aid in the diagnosis of Systemic lupus erythematosus (SLE). **For *in vitro* diagnostic use. High complexity test.**

SIGNIFICANCE AND SUMMARY

Autoimmune diseases are categorized into two general groups: One group is characterized by the production of tissue-specific autoantibodies, the other by autoimmune reactivity with normal cell nuclear and/or cytoplasmic antigens with no tissue type specificity. The latter group includes diseases such as mixed connective tissue disease, scleroderma, rheumatoid arthritis, dermatomyositis, polymyositis, Systemic lupus erythematosus (SLE) and Sjogren syndrome.[1]

Systemic lupus erythematosus is a well known chronic inflammatory illness whose clinical manifestations range from localized skin lesions to a destructive systemic disorder without cutaneous changes. SLE is characterized by remissions and exacerbations with distinct immunologic abnormalities, most notably, the presence of antinuclear antibodies. Antibodies to native DNA (anti-DNA) are of considerable interest in the diagnosis and management of patients with systemic lupus erythematosus. These autoantibodies are rarely found in patients with other rheumatic diseases, and their levels, especially those with complement fixing ability, often correlate with disease activity.[2]

The detection of elevated anti-dsDNA, complement-fixing ability and antigen avidity are important with respect to the pathogenesis of tissue damage and the clinical features of SLE.[3]

The first quantitative assay for DNA is the Farr assay.[4] The original procedure used precipitation, radio-labeled DNA and separation techniques. The difficulties in obtaining labeled DNA, the associated hazards and disposal problems of radioisotopes, and the expensive equipment required have led to the development of alternative methods.[5] Anti-DNA can also be detected utilizing the Crithidia luciliae assay. This assay requires an experienced technician in interpreting fluorescence and becomes cumbersome for large numbers of samples.

The DAI ELISA test for dsDNA antibodies has many advantages over the immunofluorescent method including a sensitive, specific assay in an easy-to-use format providing quick and efficient results. The dsDNA antigen used in this kit is purified through modified immunoaffinity adsorption methods utilizing immobilized antigen-specific human immunoglobulins.

ASSAY PRINCIPLE

The DAI dsDNA test is an Enzyme-Linked Immunosorbent Assay to detect antibodies to dsDNA antigen. Purified dsDNA antigen is attached to a solid phase microassay well. Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG, M conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.[8,9,10,11]

SPECIMEN COLLECTION & PREPARATION

Handle all blood and serum as if capable of transmitting infectious agents.[7]

1. Optimal performance of the DAIELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[12] Early separation from the clot prevents hemolysis of serum.
2. Store serum between 2 and 8°C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20 to -70°C in a non-defrosting freezer. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.

3. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
4. Do not use heat inactivate sera.
5. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[12]

MATERIALS AND COMPONENTS

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

Materials provided with the test kit

1. **Purified dsDNA antigen coated microassay plate**: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. **Serum Diluent Type II:** Ready to use. Contains ProClin® (0.1%) as a preservative. (96T: one bottle, 30 mL)
3. **Positive Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL)
4. **Calibrator:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL)
5. **Negative Control:** Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL)
6. **Horseradish-peroxidase (HRP) Conjugate:** Ready to use. Goat anti-human IgG and IgM, containing proclin (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL)
7. **Wash Buffer Type I (20X concentrate):** Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative. (96T: one bottle, 50 mL)
8. **Chromogen/Substrate Solution Type I:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)
9. **Stop Solution:** Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL)

***Note: Serum vials may contain excess volume.**

The following components are not kit lot # dependent and may be used interchangeably within the Diagnostic Automation Autoimmune Disease ELISA Kits: Chromogen/Substrate Solution Type I, and Stop Solution. Please check that the appropriate Diagnostic Automation, Inc. Reagent Type (Type I, Type II, etc.) is used for the assay.

Materials required but not provided

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH_2O), CAP (College of American Pathology) Type 1 or equivalent.[14, 15]
9. Timer capable of measuring to an accuracy of +/- 1 second (0 - 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_2O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25°C) before use. Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H_2O. Mix well.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow five (5) Control/Calibrator determinations (one Negative Control, three Calibrators and one Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #3
1B	NC	2B	Patient #4
1C	CAL	2C	Patient #5
1D	CAL	2D	Patient #6
1E	CAL	2E	Patient #7
1F	PC	2F	Patient #8
1G	Patient #1	2G	Patient #9
1H	Patient #2	2H	Patient #10

RB = Reagent Blank – well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
CAL= Calibrator
PC = Positive Control.

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. To individual wells, add 100 µL of the appropriate diluted Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21 to 25°C) for **30 minutes +/- 1 minute.**
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for

automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE:** Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25°C) for **30 minutes +/- 1 minute**.
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25°C) for **5 – 15 minutes**.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

RESULTS

1. Mean Calibrator O.D. - Calculate the mean value for the Calibrator from three Calibrator determinations. If any of the three Calibrator Values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAIfor each lot of kits. The Correction Factor is printed on the Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
4. Index Value - Calculate an Index Value for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in Step 3.

Example:

O.D.s obtained for Calibrator	= 0.38, 0.42, 0.40
Mean O.D. for Calibrator	= 0.40
Correction Factor	= 0.50
Calibrator Value	= 0.50 x 0.40 = 0.20
O.D. obtained for patient sera	= 0.60
Index Value	= 0.60/0.20 = 3.00

ANALYSIS

1. The patients' Index Values are interpreted as follows:

Index Value	Results	Interpretation
≤ 0.90	Negative	No detectable antibody to dsDNA by the ELISA test.
0.91-1.09	Equivocal	Samples should be re-tested. See number 2 below
≥ 1.10	Positive	Indicates presence of detectable antibody to dsDNA by the ELISA test.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method or test a new sample.

INTERNATIONAL UNIT CONVERSION

International unit (IU) reactivity is determined relative to the Second Generation World Health Organization (WHO) derivative. Conversion of Index values to international units is accomplished by using an exponential regression analysis. Each lot is standardized versus international units and provided with a lot specific conversion table (Conversion of International Units (IU) per mL for dsDNA IgG, IgM).

For Example:

Index Value	IU
1.0	43
1.5	64
2.0	96
2.5	145
3.0	>150

Linearity limits are 150 IU/mL. Greater than 150 IU must be reported as >150 IU/mL. See included chart for the lot specific conversion table.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met.

1. Calibrators and controls must be run with each test run.
2. Reagent Blank must be < 0.150 O.D. (Optical density) at 450 nm (when read against Blank).
3. The mean O.D. for the Calibrator should be ≥ 0.250 at 450 nm (when read against Blank).
4. The Index Values for the Positive and Negative Controls should be in their respective ranges printed on the vials. If the control values are not within their respective ranges, the test should be considered invalid and should be repeated.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS C24A for guidance on appropriate Quality Control practices.13
7. If above criteria are not met on repeat, contact DAI Technical Service.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

A study was performed using 197 patient sera obtained from outside clinical laboratories. These samples were tested using both the DAI dsDNA ELISA test and a commercially available anti-dsDNA ELISA test following the manufacturers' package inserts. Fifty-one samples were found positive by the reference ELISA test, the remaining 146 samples were negative by the ELISA. Six samples were found to be false positive and one found false negative on the DAI test as compared to the ELISA reference method. The seven discrepant samples were assayed by Crithidia IFA as the referee method. Three samples were still false positive versus the IFA method, with the other four resolving correct. Using the above data criteria, the DAI dsDNA ELISA test has a 100% sensitivity and 97.9%

specificity as compared to the results obtained on both the ELISA and IFA methods. The following data were obtained:

	Corrected ELISA and IFA				
dsDNA		+	–	Relative Sensitivity	Relative Specificity
	+	53	3	100%	97.9%
	–	0	141	53/53	141/144

Agreement = 194/197 = 98.5%

CROSS-REACTIVITY

A series of 72 patient samples each containing positive levels of antibodies to other common autoimmune markers (Scl-70, Sm, RNP, RF, Jo-1 and SS-B, SS-A, and ANA) were tested in the DAI dsDNA ELISA test. Three samples were positive on the reference ELISA test but negative on the DAIELISA and confirmed negative by IFA. Therefore, from the above data, the DAI dsDNA ELISA demonstrates no cross-reactivity to other common autoimmune markers.

INTERNATIONAL UNIT CONVERSION

The data in Table 1 illustrates dsDNA Index Values for the serially diluted Second Generation World Health Organization (WHO) derivative. The dsDNA Index Values are compared to serial dilutions of the standard serum by linear regression (exponential regression analysis). The data indicates that international units can be determined from the Index Value.

Table 1
International Unit Conversion

International Unit Standard	Index Value
Units / mL	
141	2.5
67	1.6
39	0.9
28	0.5

Linear regression compared Index Values versus international Units.

R^2 =1.000 a = 1.224 b = 3.589 Y = Index X = IU/mL

Exponential Regression Equation Calculation:

$X = \frac{(y+b)}{a}$ e^x = derived IU/mL

LIMITATIONS OF THE ASSAY

1. Only if test instructions are rigidly followed will optimum results be achieved.
2. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as washing the test strips and thorough mixing of all prepared solutions.
3. If comparisons with other methods are required, always perform both tests simultaneously.
4. Do not scratch coated wells during washing and aspiration. Wash and fill all reagents without interruption. While washing, check that all wells are filled evenly with washing solution, and that there are no residues in the wells.
5. Instructions for using appropriate photometers are to be observed; check adjustment of proper wavelength (450 nm) and reference wave length (600-650 nm optional) respectively.
6. The values obtained from this assay are intended to be an aid for diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings and other diagnostic procedures.

EXPECTED RANGES OF VALUES

Autoimmune rheumatic diseases are a group of chronic disorders, afflicting about 3% of the population. The etiology of these disorders is not elucidated but they may involve both genetic and environmental causes. Autoimmune rheumatic diseases have two common clinical and pathological features: A) a non-organ specific autoimmune condition is manifest; B) most patients exhibit some rheumatic symptoms throughout the course of their disease. It is apparent that certain systemic rheumatic diseases have distinct ANA profiles. Thus the ANA profile is helpful in the evaluation of patients with systemic lupus erythematosus (SLE), mixed connective tissue disease, scleroderma, Sjogren syndrome (SS), dermatopolymyositis, and rheumatoid arthritis (RA). Histological evidenceassociates the presence of dsDNA antibodies with the presence of systemic lupus erythematosus. Elevated antibody levels to dsDNA antigen are found in 65 - 80 % of those patients with active, untreated SLE and infrequently in other conditions.[6]

PRECAUTIONS

1. For in vitro diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.7
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type I, and Serum Diluent Type II. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21°to 25 °C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Avoid splashing or generation of aerosols. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause erroneous results.**
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.**
13. Certain reagents in this kit contain sodium azide for use as a preservative. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing proclin, sodium azide, and TMB may

be irritating. Avoid contact with skin and eyes. In case of contact, immediately flush area with copious amounts of water.

15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. Do not use Chromogen/Substrate Solution if it has begun to turn blue.
19. The concentrations of anti-dsDNA in a given specimen determined with assays from different manufacturerscan vary due to differences in assay methods and reagent specificity.

WARNING

The safety data sheet is available upon request.

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

STORAGE CONDITIONS

1. Store unopened kit between 2 and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2 and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant, and returned to storage at 2 and 8°C. If the bag is resealed with tape, the wells are stable for 30 days. If the bag is resealed with a heat sealer, the wells are stable until their labeled expiration date.
3. Store HRP Conjugate between 2 and 8°C.
4. Store the Calibrator, Positive and Negative Controls between 2 and 8°C.
5. Store Serum Diluent Type II and 20X Wash Buffer Type I between 2 and 8°C.
6. Store the Chromogen/Substrate Solution Type I between 2 and 8°C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21 to 25°C) for up to 5 days, or up to 1 week between 2 and 8°C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

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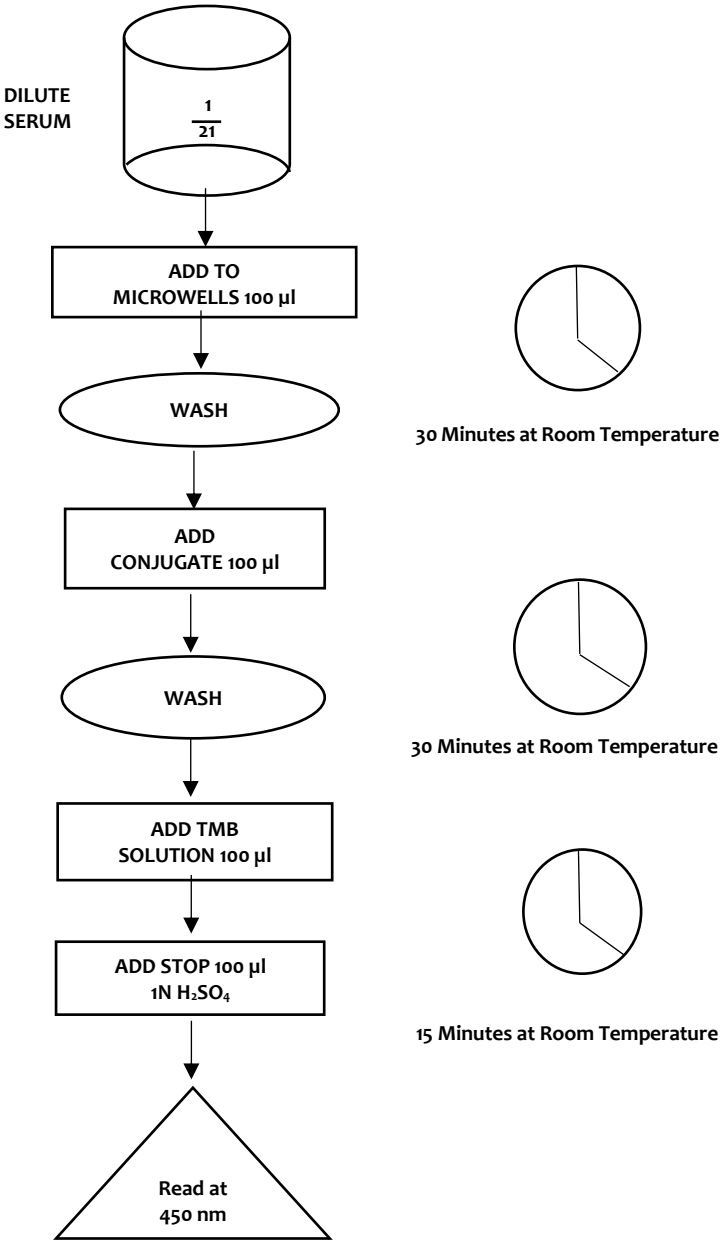
SUMMARY OF ASSAY PROCEDURE



MANUFACTURER AND BRAND DETAILS

ISO 13485: 2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

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

Date Adopted	2023-08
Brand Name	AccuDiag™
REF 2553-P1	AccuDiag™ - dsDNA ELISA
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu
Revision: 2015-03	