INTENDED USE

The **Human anti-dsDNA IgG** ELISA Kit is a high sensitivity immunoassay suitable for quantifying or titering IgG antibody activity specific for dsDNA in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

GENERAL INFORMATION

Antibodies reactive with autologous nuclear components, such as DNA and histones, can represent an autoimmune basis for pathological conditions such as systemic lupus erythematosis (SLE) in humans and in mice homozygous for the lymphoproliferation spontaneous mutation (Fas^{lpr}), a systemic autoimmunity with massive lymphadenopathy associated with proliferation of aberrant T cells, arthritis, and immune complex glomerulonephritis. These conditions include elevated levels of anti-dsDNA and other anti-nuclear antibodies (ANA) which often increase as the individual ages.

Recent investigations have focused on the role of innate immune mechanisms, including Toll-like receptors (TLRs) and TH2 immunity, responding to the damage-associated molecular patterns of dying cells as underlying cause of the anti-dsDNA type autoimmunity. These may be induced by the expanded use of biological modifiers in the drug industry, which may include immunotherapeutic antibodies, vaccines, and adjuvants.

PRINCIPLE OF THE TEST

The Human anti-dsDNA IgG ELISA kit is based on the binding of human anti-dsDNA IgG in samples to dsDNA immobilized on the microwells. Anti-dsDNA IgG antibody is detected by anti-human IgG-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate which is directly proportional to the amount of anti-dsDNA IgG present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgG antibody in samples is calculated relative to anti-DNA calibrators.

PRODUCT SPECIFICATIONS

Specificity
Purified dsDNA is used to coat the microwells; thus the assay is specific for antibodies directed to dsDNA. The anti-Human IgG HRP conjugate reacts specifically with human IgG class antibodies that bind to dsDNA on the plate. IgA, IgM, and IgE antibody would not be measured above background signals.

High Sensitivity
The dsDNA coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate anti-dsDNA IgG from background (non-antibody) signal with human serum samples diluted 1:1000.

Calibrator Values
The calibrators are dilutions of antibody reactive to dsDNA. Values are assigned as arbitrary anti-DNA activity units.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **1X Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **1X Sample Diluent** and store at 2-8°C until the kit lot expires or is used.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG-612, 0.15ml	Peroxidase conjugated anti-Human IgG in buffer with protein, detergents, and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **1X Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
dsDNA Microwell Strip Plate	5101	8-well strips (12)	Coated with dsDNA, and post-coated with stabilizers.
Anti-DNA Calibrators			
50 U/ml 200 U/ml 500 U/ml 1000 U/ml	51d53sB 51d53sC 51d53sD 51d53sE	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-DNA IgG levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Low NSB Sample Diluent Reduces non-specific binding	TBTm	30 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution. See **Assay Design**, page 3. **Not** for HRP Conjugate dilution.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:
- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

ASSAY DESIGN AND SET-UP

The **Low NSB Sample Diluent (LNSD)**, TBTm, lowers NSB even more than the 1xSD20T Diluent without diminishing true positive antibody signals, thus offering a greater discrimination between positives and non-immune samples.

Sample Collection and Handling
Culture medium, serum, and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent.

Antibody Stability
Initial dilution of serum into **1XSD20T** is recommended to stabilize antibody activity. This enhances reproducible sampling and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent,** TBTm, which provides the lowest assay background, should be at least 5 times the initial dilution; it is stable for weeks refrigerated.

Example: Initial (1:5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1:100): **10**ul initial (1:5) + **190**ul TBTm (1:100)

Assay Design
Review Calculation of Results (p. 5-7) and Limits of the Assay (below) before proceeding:

- *Select the proper sample dilutions*. Account for expected potency of positives and minimize non-specific binding (NSB) and other matrix effects. For example, non-immune samples should give net signal <0.5 OD. This is usually 1:1000 or greater dilution for human sera with normal levels of IgG and IgM. Dilute samples in **Sample Diluent (1XSD20T)** or in **Low NSB Sample Diluent (TBTm)** (see above). Note: **all samples** must be diluted in the same diluent for proper comparison – either TBTm **or** 1xSD20T.

- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations if required. Blank OD should be <0.3. **See Method A** and **B**.

- Run a set of Calibrators. Calibrators validate that the assay was performed to specifications and can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, **Method C**, has limitations. See Limits of the Assay below.

- Run a range of sample dilutions for expected higher positives that allows calculation of antibody **Titer** (when specific titer is at least 4-fold higher than non-immune)**. See Method D.**

- Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

Plate Set-up
Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).
- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of sample diluent (blank) calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well variation.

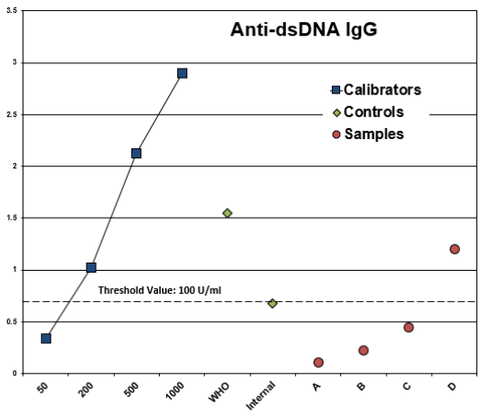
INTERPRETATION OF RESULTS

Method A. Antibody Activity Threshold Index

Compare Samples to **100 U/ml Calibrator** value or **Internal**

Control = Positive/Negative Cut-off

Example:



Results

The **sensitivity** of the assay to detect anti-dsDNA IgG is controlled so that the **100 U/ml Calibrator** value, calculated using the 50 and 200 U/ml Calibrators, represents a threshold OD for most true positives in human serum diluted to 1:1000 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of human sera with anti-dsDNA reactivity shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
100 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

WHO Reference Reagent for human antiserum reactive to dsDNA, preparation 15/174; **1** mU/ml = **320** U/ml in the assay.

Internal Control – a sample from individual(s) that represent the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the Calibrators for assessing reproducibility and to normalize between-assay variation.

Samples A,B,C,D – 3 samples (A,B,C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold;

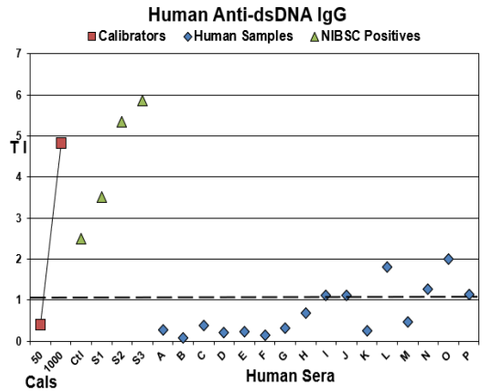
The **100 U/ml** Calibrator value can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p. 6):

- Divide each Sample OD by the 100 U/ml Calibrator OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

ASSAY PERFORMANCE

Example:

Human Serum IgG
A panel of sera from individuals of unknown history was tested for anti-dsDNA IgG alongside Positive Controls from NIBSC (1:1000 dilution in Low NSB Sample diluent). **Threshold Index** was calculated using **100** U/ml Calibrator value.



Results

Anti-dsDNA IgG: most of the samples (10) were below the suggested positive/negative 1.0 threshold; several other samples (6) were borderline or slightly positive.

Notes:

1. When the **Positive Index** is **above 5.0**, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:2000) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:500) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index** (see below) or use an **Internal Control** (Page 5).

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-treated serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sera dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

INTERPRETATION OF RESULTS (cont)

Method C. Use of a Calibrator Curve

When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay below), the anti-dsDNA activity units may be determined by interpolation from the Calibrator curve. The results may be calculated using any immunoassay software package. If software is not available, anti-dsDNA activity concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-dsDNA (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
3. The anti-dsDNA activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 1000 U/ml calibrator should be further diluted and re-assayed.

Method D. Titers from Sample Dilution Curves
The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

= **IgG Antibody Activity Units**

LIMITS OF THE ASSAY

Quantitation of Antibody in a Sample
The ELISA measures anti-dsDNA activity, a combination of antibody concentration and avidity for the dsDNA antigens. Antibodies with substantially different Ig concentrations may display similar anti-dsDNA activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of Ig (e.g., ug/ml).

Calibrator Curve Quantitation
To quantitate antibody activity from a calibrator curve (such as provided with the kit, **Method C**, the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in anti-dsDNA avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as **Method A**: ELISA value & dilution; **Method B**: a multiple of a Positive Index titer; or **Method D**: anti-dsDNA titer from a sample dilution curve (see Interpretation of Results).

Instruction Manual No. M-3100-HS

Human Anti-dsDNA IgG High Sensitivity ELISA Kit

Cat. #3100-HS, 96 tests

For Quantitation of anti-dsDNA IgG in Serum or Plasma

For in vitro research use only (RUO), not for therapeutic or diagnostic use.

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ELISA Kit Components	Amount	Part
dsDNA Coated Microwell Strip Plate	8-well strips (12)	5101
Anti-DNA Calibrator 50 U/ml	0.65 ml	51d53sB
Anti-DNA Calibrator 200 U/ml	0.65 ml	51d53sC
Anti-DNA Calibrator 500 U/ml	0.65 ml	51d53sD
Anti-DNA Calibrator 1000 U/ml	0.65 ml	51d53sE
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG-612
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-3100-HS