

Instructions for use

For *in vitro* diagnostic use only

IgD Quant EIA

A solid-phase enzyme immunoassay for the detection of immunoglobulin D in human serum or plasma.



Product no. 6400 100 (Microstrips®, 96 wells)

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INTENDED USE

Ani Lab systems' IgDquant EIA test is a simple indirect solid-phase immunoassay enabling the precise quantification of immunoglobulin D (IgD) present in human serum or plasma samples.

INTRODUCTION

Discovered in 1965, IgD is a unique immunoglobulin with a concentration in serum far below those of IgA, IgG and IgM but much higher than that of IgE. IgD is located on the surface of many B cells and is the major antigen receptor isotype on the surface of most peripheral B cells, where it is coexpressed with IgM¹.

Hyper Immunoglobulin D Syndrome (HIDS) has been reported as a major cause of elevated level of IgD. HIDS is found in patients with unexplained periodic fever and joint disease. High level of IgD is also found in IgD myeloma, associated with heavy Bence Jones proteinuria, diverse infections (tuberculosis, leprosy, aspergillosis), AIDS, rheumatoid polyarthritis, Hodgkin's disease, diabetes, cirrhosis, Mediterranean family fever, tobacco smoking and pregnancy^{1,2}.

Little is known about the normal function of IgD, and few clinical signs or symptoms are associated with its absence. Immunoglobulin D (IgD) deficiency is a defect of humoral

immunity that is characterized by abnormally low serum levels of IgD. It has been proven in mice that IgD may substitute for some functions of IgM when IgM is absent. In US, approximately 10 % of normal blood donor population had low or undetectable IgD levels. The incidence is not correlated to age or gender.

Traditionally, IgD level in serum has been measured with the other classes of immunoglobulin since low levels of IgD may be associated with the presence of other immune disorders. In case of IgD deficiency discovery, the patient should be referred to an allergist or clinical immunologist to help exclude other more serious related conditions³. Because of the susceptibility to proteolysis, radial immunodiffusion may overestimate IgD level, whereas turbidometry is not sensitive enough for low concentration of proteins, therefore Ani Lab systems' IgDquant EIA offers a robust and reliable tool for the quantification of human IgD in serum or plasma.

PRINCIPLE OF THE TEST


The principle of the Ani Lab systems' IgDquant EIA kit is based on indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme. The assay proceeds according to the following reactions.

Immunoglobulins D from the patient sample bind to capturing anti-human IgD antibodies attached to the polystyrene surface of the Microstrip® wells. Residual patient sample is removed by washing and horseradish peroxidase conjugated anti-human IgD is added. Unbound conjugate is washed off and a colorless enzyme substrate (H₂O₂) containing the chromogen (TMB, Tetramethylbenzidine, a non-mutagenic chromogen for horseradish peroxidase) is added. The enzyme reaction with the chromogen results in a coloured end product. The colour formation reaction is terminated by adding acid (H₂SO₄). The color intensity is directly proportional to the concentration of IgD in a patient sample.


KIT CONTENTS

Note:

- Reagents are stored between +2°C and +8°C.
- The expiration date is printed on each component label and on the package. Do not use reagents after the expiration date.
- Avoid unnecessary exposure to light. This is merely a precaution. The light sensitive reagents are the conjugate and the TMB-substrate solution, the latter one is packaged in non-transparent plastic vials for protection.

- 1 MICROSTRIPS®, 12 x 8 wells
Anti-IgD Coated Microstrips®.
- 2 SAMPLE DILUENT, 100 ml
Phosphate buffered saline with proprietary additives, a blue colouring reagent, and 0.05 % Bronidox® as preservative.
- 3 CALIBRATORS A -F, 0.55 ml 
Ready to use calibrators containing 0.05 % Bronidox® as a preservative and a red coloring reagent.
A = 0 mg/L, B = 15 mg/L, C = 50 mg/L

D = 100 mg/L, E = 250 mg/L, F = 500 mg/L
Potential biohazardous material.

- 4 CONTROLS G – H, 0.55 ml 
Diluted human serum with 0.05 % Bronidox® as a preservative. Potential biohazardous material.
- 5 CONJUGATE, 25 ml
Buffered salt solution with proprietary additives, a red colouring reagent, horseradish peroxidase conjugated anti-human IgD (goat) with 0.1% N-Methylisothiazolone as preservative
- 6 WASHING SOLUTION, 50 ml
20-fold concentrated citrate buffered saline, with proprietary additives, and 0.05 % Bronidox® as preservative.
- 7 TMB-SUBSTRATE SOLUTION, ready to use, 18 ml
Citrate buffered solution of 3,3',5,5'-Tetramethylbenzine and hydrogen peroxide with proprietary additives and 0.01% Kathon CG as preservative.
- 8 STOPPING SOLUTION, 25 ml
0.45 M H₂SO₄

INCUBATION COVERS, 2 pcs

REAGENT BASINS, 6 pcs

REAGENT PREPARATION

Reagent	Preparation	Stability of opened/diluted reagents (+2°C to +8°C)
1 Coated Microstrips®	Ready for use	9 months *)
2 Sample diluent	Ready for use	9 months *)
3-4 Calibrators / Controls	Ready for use	9 months *)
5 Conjugate	Ready for use	9 months *)
6 Washing solution concentrate (20x)		9 months *)
Washing solution	Dilute the concentrate 1+19 (1:20) in distilled water	1 month at +4°C or 1 week at room temperature
7 TMB- Substrate solution	Ready for use	9 months *) Discard unused reagent from the reaction basin. A deep blue color present in the substrate indicates that the solution has been contaminated and must be discarded.
8 Stopping solution	Ready for use	9 months *)

- *) Once the Microstrip® foil-package is opened it should be resealed tightly with the desiccant: fold the opened end a few times and seal air-tightly with tape over the whole length of the opening. The stability of the opened reagents is the maximum only if they are stored properly at +2°C to +8°C. High environmental temperature and contamination may decrease the stability.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water, preferably sterile.
- Graduated cylinders for reagent dilution.
- Vials to store the diluted reagents.
- Precision pipettes (one channel e.g. 1-10 µl, 5-50 µl, 20-200µl, 100-1000 µl ranges and multi-channel 50-300 µl)
- Paper towels or absorbent paper.
- Timer, 90 min range.
- Microplate incubator
- Microplate photometer, 450 nm
- Microplate washer (not compulsory)
- Sodium hypochlorite solution, free available chlorine 50-500 mg/l.
- Disposable gloves.

PRECAUTIONS

For *in vitro* diagnostic use only.

Warning - POTENTIAL BIOHAZARDOUS MATERIAL:

Each donor unit used in the preparation of the calibrators/controls sera in the kit has been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. Because no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, these calibrators and controls as well as specimens should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes for Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1999.

Discard all materials and specimens as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free available chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal. Reusable glassware must be disinfected, washed out and rinsed free of detergents.

Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. Stopping solution (vial 8) contains 0.45 M sulphuric acid, avoid contact with skin and eyes.

Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.

Do not mix or interchange reagents from different lots. Do not interchange vial caps.

Once the assay has been started, all subsequent steps should be performed without interruption. Do not let the wells dry once the assay has been started.

Do not reuse a Microstrip® even if some wells were not used.

SPECIMEN COLLECTION AND HANDLING

Serum and plasma samples should be refrigerated (+4°C) after collection or, if the test cannot be performed within 48 hours, frozen (-20°C or preferably -70°C).

Do not use sodium azide as preservative because it inactivates horseradish peroxidase.

Heat inactivation of serum or plasma (+56°C, 30 min.) may cause non-specific results.

TEST PROCEDURE

PRELIMINARY PREPARATIONS

- **Bring the reagents and Microstrips® to room temperature (+20°C to +25°C) before starting the assay.**
- Prewarm the incubator to +37°C.

Prestep Dilute samples 1:250 in sample diluent ¹⁻².

STEP I

- pipette **100 µl** of each calibrators (3A to 3F) and controls (4G and 4H) in wells A1 to H1 ¹⁻²

- pipette **5 µl** of each diluted samples and **95 µl** of sample diluent starting from well A2 ¹⁻².

Cover the plate and incubate **60 min** (+/- 5 min) **at +37°C** (+/-1°C)

Wash **5 x 300-400 µl/well** ³.

STEP II

Add **100 µl** conjugate solution (vial 5) into each well ¹.

Cover the plate and incubate 60 min (+/- 5 min) **at +37°C** (+/-1°C)

Wash **5 x 300-400 µl/well** ³.

STEP III

Add **100 µl** TMB-substrate solution (vial 7) into each well ¹.

Incubate **30 min** (+/- 2 min) **at RT** (+20°C - +25°C) **in dark**

STEP IV

Add **100 µl** stopping solution (vial 8) into each well. Mix well.

Measure immediately at **450 nm**

NOTES:

The use of an 8-channel pipette device is recommended for improved efficiency and precision.

1. **Avoid contamination:** When removing aliquots from the reagent vials, use aseptic technique to avoid contamination. Use a new pipette tip for each sample. **Pour needed amount of the conjugate and substrate solutions into a disposable reagent basin. Discard any unused solutions; do not pour it back to the vials.** For this purpose the kit includes 6 disposable reagent basins.

Do not touch the walls of the wells with pipette tips when adding TMB-substrate.

2. Dilute the specimen 1:250 in sample diluent (4 µl serum or plasma sample and 996 µl of sample diluent). **Do not dilute calibrators or controls.**

3. Washing may be performed manually or with a washer. After the washing step tap the inverted Microstrip® a few times on the paper towel.

RESULTS

Quality Control Values

Before calculating the results, make sure that the absorbance values obtained for the calibrators and the controls fall within the Quality Control guidelines indicated in Table 1.

If the calibrators and the controls do not give expected values, the results are invalid and the specimens should be retested.

Table 1. Quality control values

QC Sample	Expected absorbance units
Calibrator 3A	< 0.170
Calibrator 3F	1.400 – 2.700
Abs. ratio 3D / 3C	1.5 – 1.9
Abs. ratio 3F / 3C	4.0 – 6.2
	Expected concentration values (mg/L)
Control 4G	4 – 25
Control 4H	56 - 113

Calculation of the Results

Manual calculation:

Draw a calibration curve on graph paper, where the mean Abs is plotted on the Y-axis and calibrator concentrations on the X-axis.

Read the IgDquant controls and specimens from the calibration curve.

Automatic calculation:

If automatic data processing is available, Cubic Spline curve should be generated from the mean absorbance of calibrators values with a lin-lin axis scaling.

Samples showing signals above the highest standard should be diluted further in sample diluent.

Interpretation of the Results

The test will give quantitative values for IgD present in specimen. It is recommended that each laboratory establishes its own range of values according to status of the patients. The analyte ranges should be determined individually for both serum and plasma samples.

However, three independent studies analysing adult serum samples concluded that the Cut Off of IgD concentration for healthy patient is 140 mg/L^{4, 5, 7}.

Special attention should be paid to neonates, since the average concentration for neonates has been reported to be much lower⁷.

All concentrations in the kit are given as mg/L.

Samples exhibiting concentration above the highest calibrator (3F = 500 mg/L) should be further diluted in sample diluent.

LIMITATIONS OF THE PROCEDURE

Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical condition and other laboratory methods.

PERFORMANCE CHARACTERISTICS

Reproducibility

Within run reproducibility

	replicates n	Mean abs.	CV%	Conc. mg/L	CV%
Sample 1	12	0,178	2,8	15	5,9
Sample 2	12	0,655	4,9	100	6,1
Sample 3	12	1,391	3,9	250	5,2
Sample 4	12	2,153	3,3	500	5,7
Sample 5	12	0,171	3,8	14	8,4
Sample 6	12	0,530	2,8	76	3,7

Between run reproducibility

	run n	Mean Abs	CV %	Conc. mg/L	CV%
Sample 7	16	0,176	3,8	16	7,4
Sample 8	16	0,604	3,2	89	7,8
Sample 9	16	0,132	3,4	8	13,2
Sample 10	16	0,569	8,0	81	12,6

Summary of the evaluation studies

Ani Lab systems' IgD Quant EIA was evaluated in a university hospital laboratory in Finland on 50 samples with high and low concentrations. The results were compared to the concentrations obtained with nephelometry and with electrophoresis. It appeared that 36% of the samples were not within the detection range of the nephelometry method. Ani Lab systems' IgD Quant EIA allowed determination of IgD concentration for 100 % of the tested samples. It gave a concentration in agreement with the protein peak area calculation of the electrophoresis profile. In addition, the turbidity of some samples did not interfere with the EIA while it did with the nephelometry.

53 % of 57 samples sent for determination by radial immunodiffusion (RID) in a private laboratory had IgD level under the detection limit of RID. Out of those 57 samples, 18 samples were chosen (11 undetectable + 5 normal + 2 very high) and analyzed with Ani Lab systems' IgD Quant EIA. 100 % of the samples gave detectable concentration with Ani Lab systems' IgD Quant EIA. The degradation of IgD in some samples may explain the high concentration observed with RID but not with EIA. In fact it is known that degradation interferes strongly with RID but it does not hinder the proper quantification with EIA.

Analytical specificity

Comparison with other analytical methods:

Ani Lab systems' IgD EIA was compared to two other analytical methods, namely radial immunodiffusion and nephelometry using serum samples. The following correlation factors were obtained:

Correlation factors	
EIA – Radial ImmunoDiffusion	0.92
EIA - Nephelometry	0.99

Cross reactivity

The cross reactivity with other immunoglobulin classes has been assessed with range of purified IgG, IgA, IgM or IgE varying from 0 to 3 g/L. For each class the cross reactivity was below 1 %.

Analytical sensitivity

The sensitivity, or detection limit, was determined as the concentration of IgD at two standard deviation from the zero calibrator (3A) and corresponds to 0.1 mg/L of serum or plasma.

TROUBLE SHOOTING

3A calibrator has too low/high absorbance value	
Cause/Error	Remedy
1. Contamination, spills from other wells	Avoid contamination
2. Conjugate contamination	

3F calibrator has too low absorbance value	
Cause/Error	Remedy
1. Degradation of calibrators	Respect storage conditions, avoid contamination

All absorbance values are very high	
Cause/Error	Remedy
1. TMB-substrate solution is contaminated	Use clean containers
2. Washing solution concentrate has not been diluted correctly	Should be diluted 1:20 (1+19)
3. Poor washing	Check your washer
4. Contaminated solution containers	Use clean containers

5. Deterioration of reagents	Use aseptic technique. Do not pour used reagents back to vials
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All absorbance values are very low	
Cause/Error	Remedy
1. Incubation temperature is too low	Incubate at 37°C (± 1°C). The heating efficiency of incubators varies widely. The incubator with efficient and even warming is preferred.
2. Contamination of conjugate by human serum or plasma	One microliter of human serum/plasma is enough to inhibit as much as 1 liter of the conjugate. Never pour used conjugate back to vial!
3. The reagents have not been warmed up to room temperature	Should be +20°C - +25°C when starting the assay
4. Once opened microtiter plate foil package has not been resealed tightly and stored properly with desiccant	See instructions in REAGENT PREPARATION
5. TMB-substrate solution is exposed to direct sunlight	Avoid unnecessary exposure to light
6. Deterioration of reagents	Use aseptic technique. Do not pour used reagents back to vials

Poor precision	
Cause/Error	Remedy
1. Liquid handling devices are not properly calibrated	Check calibration of the pipetting device
2. Improper washing due to contamination of washing tips head	Clean regularly tips of the washing head
3. The plate is allowed to stay too long after washing (drying of the plate)	Follow strictly the kit instructions
4. Uneven warming of the plate	Service the incubator in use
5. Sample serum/plasma is not mixed properly with sample buffer	While pipetting mix the sample with sample buffer
6. Stopping solution has not been mixed properly before measurement	Mix the plate before measuring

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RELATED PRODUCTS AND ORDER INFORMATION:

Product number	Product description
6400 100	IgDquant EIA

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