

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgM concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Related ELISA kits

Catalog#	ProdDescription
400-100-DGG	Dog IgG ELISA Kit, 96 tests, Quantitative
400-170-DGM	Dog IgM ELISA Kit, 96 tests, Quantitative
400-180-DGA	Dog IgA ELISA Kit, 96 tests, Quantitative
400-190-DGE	Dog IgE ELISA Kit, 96 tests, Quantitative
400-195-DGT	Dog Transferrin ELISA Kit, 96 tests, Quantitative

Instruction Manual No. M-400-170-DGM

Dog IgM ELISA Kit

Cat. No. 400-170-DGM, 96 Tests

For Quantitative Determination of Dog Immunoglobulin M in serum, plasma or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.



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INTENDED USE

The Alpha Diagnostics Int'l Dog IgM ELISA Kit is an in vitro immunoassay for the quantification of IgM circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of dog cells.

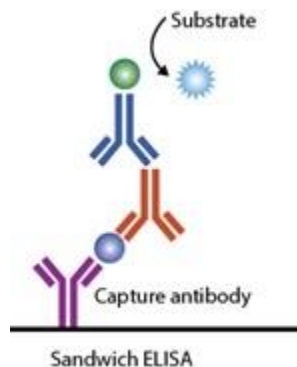
RESEARCH USE OF THE TEST

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of dog immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrums, ascites, tears and mucosa of linings of the gut, respiratory or urigenital tracts.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hypergammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to the concomitant determination of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassays measure dog IgG, IgA and IgM with high sensitivity, that allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Each assay is Ig class specific, such that all IgG and IgA subclasses are reliably quantified in essentially any specimen, freshly obtained and/or suitable stored. Expected performance of each kit relative to precision, linearity and normal values is presented for guidance of use.

PRINCIPLE OF THE TEST



The Dog IgM ELISA kit is based on the binding of dog IgM in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgM present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgM in samples and control is calculated from a curve of standards containing known concentrations of dog IgM.

STORAGE AND STABILITY

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

PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgM, and have essentially no reactivity with IgG, IgA, or any other dog serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, rat, hamster, guinea pig, bovine, horse, sheep, goat, pig, cat, rabbit or chicken; also 10% neonatal bovine serum.

Normal Range

IgM levels in dogs will vary with breed, environment and age, with little variation with sex. Normal adult IgM levels have been reported to be in the 1-3 mg/ml range. Expected levels should be established by the laboratory for the population to be tested.

Precision

Samples containing low, medium and high concentrations of IgM were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

IgM concentrations were measured with good within-assay (3.7 to 4.6 %CV) and between-assay (2.6 to 5.0 %CV) reproducibility.

Sample	IgM ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	134	4.2	2.6
Mid Sample	299	3.7	2.8
High Sample	630	4.6	5.0

Linearity of Dilution

Three (3) mixed male and female pooled stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 96 to 99%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Dog Serum A	1:4k	690	2.76	96 %
	1:32k	94	3.01	
Dog Serum B	1:2.4k	870	2.09	97 %
	1:19.2k	116	2.23	
Dog Serum C	1:4k	651	2.60	99 %
	1:16k	166	2.66	

CALCULATION OF RESULTS

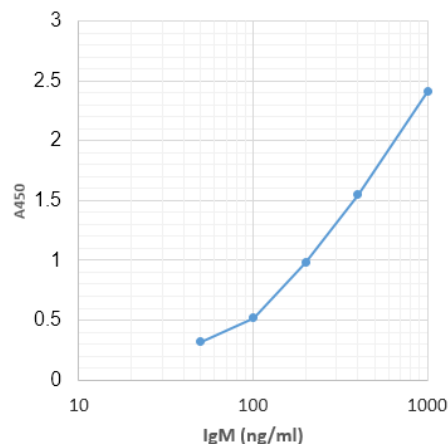
- The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgM concentrations may be determined as follows:
- Calculate the mean OD of duplicate samples.
- On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgM (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
- The IgM concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by the dilution factor of each sample.
- Samples producing signals higher than the 1000 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	IgM ng/ml
A1, A2	Negative Diluent Control	0.10	0
B1, B2	50 ng/ml Standard	0.32	50
C1, C2	100 ng/ml Standard	0.52	100
D1, D2	200 ng/ml Standard	0.98	200
E1, E2	400 ng/ml Standard	1.55	400
F1, F2	1000 ng/ml Standard	2.41	1000
G1, G2	Positive Serum Control [Value: 210 - 390 ng/ml]	1.26	290
H1, H2	Sample [Diluted 1:20k] Calculated: 20k-fold dilution x 108 ng/ml = 2.16 mg/ml in serum	0.56	108

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
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 Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
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 Working Wash Solution and store at RT until kit is used entirely.
Anti-Dog IgM - HRP Conjugate Concentrate (100x) Part No. 400-174, 0.15ml	Peroxidase conjugated anti-dog IgM in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Dog IgM Microwell Strip Plate	400-171	8-well strips (12)	Coated with purified anti-dog IgM antibodies.
Dog IgM Standards			
50 ng/ml	400-173B	0.65 ml	Five (5) vials, each containing dog serum with designated IgM concentrations; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
100 ng/ml	400-173C	0.65 ml	
200 ng/ml	400-173D	0.65 ml	
400 ng/ml	400-173E	0.65 ml	
1000 ng/ml	400-173F	0.65 ml	
Positive Control [IgM] range on label	400-172	0.65 ml	Dog IgM of stated concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
TMB Solution	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Antibody-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% 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.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site for Proclin-300 (0.1% v/v in standards, and assay buffers).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

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 Working Sample Diluent. If samples will not be assayed immediately, stored refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to lab temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 5 to 20k-fold are appropriate for most normal dog sera. For accuracy, two dilution steps are recommended, as follows:

- 1) 5ul serum + 995ul diluent = [1:200],
- 2) 5ul [1:200] + 495ul diluent = [1:20k].

DO NOT dilute the Standards or Positive Control Serum.

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. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard 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 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation

[100ul - 60min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.

Wash as follows: aspirate or dump the well contents, add 250-300ul Working Wash Solution, then immediately aspirate or dump the solution. Repeat 3 times = 4 washes. Pat the wells 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.

3. 2nd Incubation

[100ul - 30min; 5 washes]

- Add 100ul of diluted Anti-dog IgM-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation

[100ul - 15min]

- 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).

5. 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.

6. 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 background.