

Monkey IgA

ELISA Kit Cat. No. 7010

For Quantitative Determination of Monkey Immunoglobulin A in Fluids



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INTENDED USE

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

RESEARCH USE OF THE TEST

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of monkey 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 urogenital 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 monkey 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, recovery and linearity of dilution is presented for guidance of use.

PRINCIPLE OF THE TEST

The Monkey IgA ELISA kit is based on the binding of monkey IgA 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 IgA 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 IgA in samples and control is calculated from a curve of standards containing known concentrations of IgA.

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.

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-Monkey IgA - HRP Conjugate Concentrate (100x) Part No. 7014, 0.15ml | Peroxidase conjugated anti-Monkey IgA 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-Monkey IgA Microwell Strip Plate | 7011 | 8-well strips (12) | Coated with purified anti-Monkey IgA antibodies. |
| Monkey IgA Standards | | | |
| 25 ng/ml | 7013B | 0.65 ml | Five (5) vials, each containing monkey serum with designated IgA concentrations; diluted in buffer with protein, detergents and ProClin 300 as stabilizers. |
| 50 ng/ml | 7013C | 0.65 ml | |
| 100 ng/ml | 7013D | 0.65 ml | |
| 200 ng/ml | 7013E | 0.65 ml | |
| 500 ng/ml | 7013F | 0.65 ml | |
| Positive Control [IgA] range on label | 7012 | 0.65 ml | Monkey IgA 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 pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Monkey IgA-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.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately, stored refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles. The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Monkey serum may contain zoonotic, cross-species infectious material. Always wear gloves when handling serum-containing samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Standards, Controls, Sample Diluent, and Anti-monkey IgA-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.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

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 Control is provided with the kit, assigned with an IgA 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.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

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

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 40k-fold are appropriate for most normal monkey sera. For accuracy, two dilution steps are recommended, as follows:

- 1) 5ul serum + 995ul diluent = [1:200],
- 2) 5ul [1:200] + 995ul diluent = [1:40k].

DO NOT dilute the Standards or Control.

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, before sample addition, to each well and let stand about 5 minutes.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation

[100ul – 60 min; 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 wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation

[100ul – 30 min; 5 washes]

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

4. 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, assuring the top standard does not surpass 2 OD.

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.

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, IgA concentrations may be determined as follows:

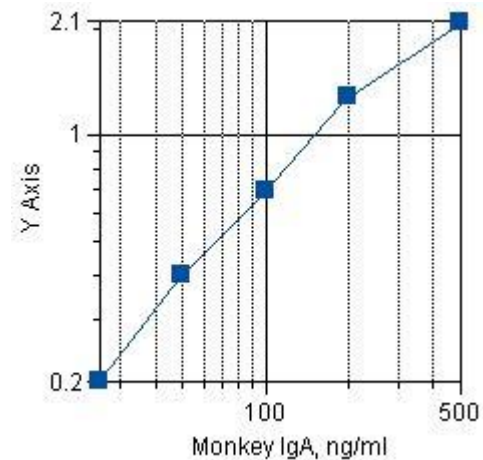
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgA (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The IgA concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 500 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 | IgA ng/ml |
|--------|---|---------|-----------|
| A1, A2 | Negative Diluent Control | 0.01 | 0 |
| B1, B2 | 25 ng/ml Standard | 0.20 | 25 |
| C1, C2 | 50 ng/ml Standard | 0.40 | 50 |
| D1, D2 | 100 ng/ml Standard | 0.69 | 100 |
| E1, E2 | 200 ng/ml Standard | 1.28 | 200 |
| F1, F2 | 500 ng/ml Standard | 2.08 | 500 |
| G1, G2 | Positive Control [Value: 105 - 195 ng/ml] | 0.98 | 144 |
| H1, H2 | Sample [Diluted 1:10k] Calculated: 10k-fold dilution x 212 ng/ml = 2.12 mg/ml in serum | 1.34 | 212 |

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



PERFORMANCE CHARACTERISTICS

Specificity

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

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

Normal Range

Assay of IgA in stored sera from eleven (11) individual Rhesus, Cynomolgous and Baboons ranged from 6 to 23 mg/ml (median = 14 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgA, representing 3 different species, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=4-5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

IgA concentrations were measured with good within-assay (4.2 to 9.3 %CV) and between-assay (6.1 to 16.2 %CV) reproducibility.

| Sample | IgA ng/ml | Intra-assay %CV | Inter-assay %CV |
|-------------|--------------|--------------------|--------------------|
| Cynomolgous | 194 | 4.8 | 16.2 |
| Rhesus | 258 | 4.2 | 9.0 |
| Baboon | 49 | 9.3 | 6.1 |

Linearity of Dilution

Four (4) individual stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery of the Monkey and Chimpanzee sera ranged from 91 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

| Sample | Dilution | Assay Value ng/ml | Serum Value mg/ml | Concordance |
|-------------|----------|----------------------|----------------------|--------------|
| Cynomolgous | 1:60k | 432 | 25.9 | 98 % |
| | 1:240k | 104 | 25.0 | |
| Rhesus | 1:60k | 305 | 18.3 | 91 % |
| | 1:240k | 64 | 15.4 | |
| Baboon | 1:60k | 260 | 15.6 | 92 % |
| | 1:240k | 56 | 13.4 | |
| Chimp | 1:6k | 403 | 2.42 | 100 % |
| | 1:48k | 50 | 2.40 | |

PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery

High and low concentrations of purified monkey IgA were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 94 to 139%, indicating accurate quantification of IgA in monkey serum.

| Sample | Expected ng/ml | Observed ng/ml | Observed/ Expected |
|----------------------|-------------------|-------------------|-----------------------|
| High IgA Spike | | 1777 1115* | |
| + Baboon, 661 ng/ml | 2438 | 2666 | 109 |
| + Rhesus, 1121 ng/ml | 2898 | 3242 | 112 |
| + Cyno A, 224 ng/ml | 1339* | 1355* | 101 |
| Low IgA Spike | | 406 | |
| + Baboon, 661 ng/ml | 1067 | 1479 | 139 |
| + Rhesus, 1121 ng/ml | 1527 | 1824 | 119 |
| + Cyno B, 2399 ng/ml | 2805 | 2645 | 94 |

| ELISA KIT COMPONENTS | Amount | Part No. |
|---------------------------------------|--------------------|----------|
| Anti-Monkey IgA Microwell Strip Plate | 8-well strips (12) | 7011 |
| Monkey IgA Positive Control | 0.65 ml | 7012 |
| Monkey IgA Standard 25 ng/ml | 0.65 ml | 7013B |
| Monkey IgA Standard 50 ng/ml | 0.65 ml | 7013C |
| Monkey IgA Standard 100 ng/ml | 0.65 ml | 7013D |
| Monkey IgA Standard 200 ng/ml | 0.65 ml | 7013E |
| Monkey IgA Standard 500 ng/ml | 0.65 ml | 7013F |
| Anti-Monkey IgA HRP Conjugate (100x) | 0.15 ml | 7014 |
| Sample Diluent Concentrate (20x) | 10 ml | SD-20T |
| Wash solution Concentrate (100x) | 10 ml | WB-100 |
| TMB Substrate | 12 ml | 80091 |
| Stop Solution | 12 ml | 80701 |
| Product Manual | 1 ea | M-7010 |