ab190530 –
IgA Horse ELISA Kit

Instructions for Use

For the quantitative measurement of IgA in horse serum and plasma.

This product is for research use only and is not intended for diagnostic use.

Version 1b Last Updated 24 November 2020

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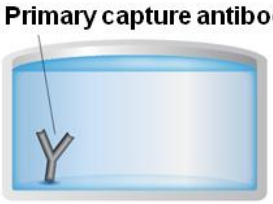
1. BACKGROUND

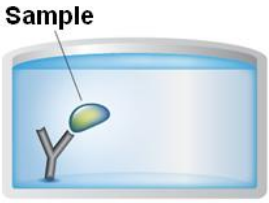
Abcam's IgA Horse ELISA kit is an *in vitro* enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of IgA in horse serum and plasma.

In this assay the IgA present in samples reacts with the anti-IgA antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-IgA antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound IgA. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgA in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgA in the test sample. The quantity of IgA in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

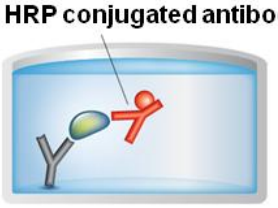
2. ASSAY SUMMARY



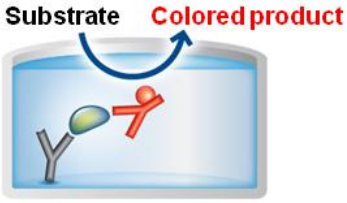
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate at room temperature



Aspirate and wash each well. Add Chromogen Substrate Solution to each well. Immediately begin recording the color development

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
IgA Horse Antibody pre-coated 96 well microplate (12 x 8 well strips)	96 wells	+2-8°C
IgA Horse Calibrator (Lyophilised)	1 vial	-20°C
IgA Horse 5X Diluent	50 mL	+2-8°C
20X Wash Buffer Concentrate	50 mL	+2-8°C
IgA Horse HRP Conjugate	1 vial	+2-8°C
TMB Substrate	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions.
- Test tubes.
- Microtitre washer/aspirator.
- Distilled or Deionized H_2O.
- Microtitre Plate reader.
- Assorted glassware for the preparation of reagents and buffer solutions.
- Timer.

7. LIMITATIONS

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
- Do not mix or substitute reagents with those from other lots or sources.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 IgA Horse Diluent

The diluent solution is supplied as 5X Diluent Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O). The 1X Diluent Solution is stable for at least one week from the date of preparation and should be stored at 2-8°C.

9.2 Wash Buffer

The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at room temperature (16-25°C) or at 2-8°C.

9.3 1X IgA Horse HRP Conjugate

Calculate the required amount of 1X IgA Horse HRP Conjugate solution for each microtitre plate test strip by adding 10 µL IgA Horse HRP Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix gently but thoroughly. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 **The lyophilized IgA Horse Calibrator should be reconstituted with distilled or de-ionized water as specified on the vial.** The concentration listed on the vial is the concentration after it is reconstituted with this volume. The reconstituted calibrator should be aliquoted and stored at -20°C if future use is required. Avoid multiple freeze-thaw cycles.

As an example: Add 1.0 mL of distilled or de-ionized water to reconstitute the lyophilized IgA Horse Calibrator to a concentration of 226.1 µL /mL

10.2 Label eight tubes A and # 1-7.

10.3 Prepare a (1/100 dilution) Standard A by adding the appropriate volume of 1X Diluent Solution and reconstituted IgA Horse Calibrator to tube A. Mix thoroughly but gently.

As an example: If the IgA Horse Calibrator is reconstituted to a concentration of 226.1 µg/mL, then to generate a 2,261 ng/mL Standard A, add 5 µL of the 226.1 µg/mL IgA Horse Calibrator to 495 µL 1X Diluent Solution.

10.4 Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution and **Standard A** (derived below) to tube #1.

*Example:

NOTE: THIS EXAMPLE IS FOR DEMONSTRATION PURPOSES ONLY. PLEASE REMEMBER TO CHECK YOUR CALIBRATOR VIAL FOR THE ACTUAL CONCENTRATION OF CALIBRATOR PROVIDED.

C_S = Starting concentration of **Standard A** (variable e.g. 2,261 ng/mL)

C_F = Final concentration **Standard #1** (500 ng/mL)

V_A = Total volume of **Standard A** to dilute (e.g. 180 µL)

V_D = Total volume of 1X Diluent Solution required to dilute **Standard A** to prepare **Standard #1**

V_T = Total volume of **Standard #1**

D_F = Dilution factor

Calculate the dilution factor (D_F) between **Standard A** and **Standard #1** final concentration:

$$Cs/C_F = D_F$$

$$2{,}261 / 500 = 4.52$$

Calculate the final volume V_D required to prepare the **Standard #1** at 500 ng/mL

$$V_A * D_F = V_T$$

$$180 * 4.52 = 814\ \mu L$$

$$V_D = V_T - V_A$$

$$V_D = 814 - 180 = 634\ \mu L$$

To prepare **Standard #1** in tube #1, add 180 µL of **Standard A** to 634 µL of 1X Diluent Solution to obtain a concentration of 500 ng/mL. Mix thoroughly and gently.

10.5 Add 300 µL 1X Diluent Solution into tube # 2-6.

10.6 Prepare **Standard #2** by adding 300 µL **Standard #1** to tube #2. Mix thoroughly and gently.

10.7 Prepare **Standard #3** by adding 300 µL from **Standard #2** to tube #3. Mix thoroughly and gently.

10.8 Using the table below as a guide to prepare further serial dilutions.

10.9 **Standard #7** is a Blank control (0 ng/mL) add 600 µL 1X Diluent Solution into tube # 7.

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
A	See step 10.3				
1	See step 10.4				
2	300 Std. #1	300	600	500	250
3	300 Std. #2	300	600	250	125
4	300 Std. #3	300	600	125	62.5
5	300 Std. #4	300	600	62.5	31.25
6	300 Std. #5	300	600	31.25	15.63
7 (Blank)	N/A	600	600	0	0



11. SAMPLE COLLECTION AND STORAGE

11.1 **Serum** – Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

11.2 **Plasma** – For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results.

Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Precautions**

  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- **Additives and Preservatives**

  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

12. SAMPLE PREPARATION

General Sample information:

The assay for quantification of IgA in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/10,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/10,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 sample by transferring 5 µL to 495 µL of 1X diluent. You now have a 1/10,000 dilution of your sample. Mix thoroughly at each stage.

13. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Pipette 100 µL of each standard, including zero control, in duplicate, into pre designated wells.

14.2 Pipette 100 µL of sample (in duplicate) into pre designated wells.

14.3 Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

14.4 Following incubation, aspirate the contents of the wells.

14.5 Completely fill each well with appropriately diluted 1X Wash Buffer and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

14.6 Pipette 100 µL of appropriately diluted 1X IgA Horse HRP Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.

14.7 Wash and blot the wells as described in 14.4 - 14.5.

14.8 Pipette 100 µL of TMB Substrate into each well.

14.9 Incubate in the dark at room temperature for precisely ten (10) minutes.

14.10 After ten minutes, add 100 µL of Stop Solution to each well.

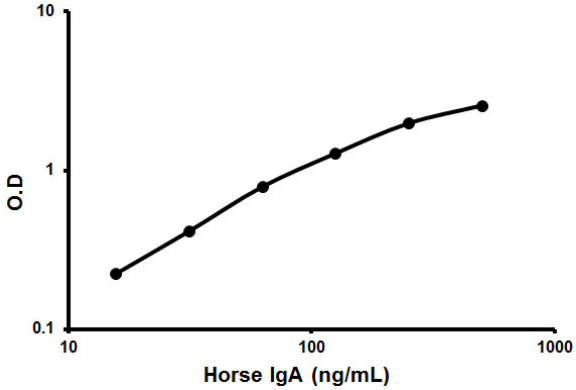
14.11 Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

15. CALCULATIONS

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in IgA Horse Diluent and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

16. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements	
Concentration (ng/mL)	**Mean O.D.**
15.63	0.223
31.25	0.413
62.5	0.787
125	1.277
250	1.978
500	2.56

17. TYPICAL SAMPLE VALUES

SENSITIVITY –

Calculated minimum detectable dose = 3.654 ng/mL

RECOVERY –

Control Serum Recovery = > 85%

PRECISION –

	Intra-Assay	Inter-Assay
%CV	<10	<10

18. INTERFERENCES

These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure.

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

20. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

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