ab137980 Human IgA ELISA Kit

For the quantitative measurement of human IgA in plasma, serum, urine, saliva, milk, cerebrospinal fluid and cell culture supernatants. This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab137980
(use abcam.cn/ab137980 for China, or abcam.co.jp/ab137980 for Japan)

Materials Supplied and Storage

Store kit at +4°C immediately upon receipt, apart from the Standard, SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components below.

Item	Quantity	Storage Condition
IgA Microplate (12 x 8 wells)	96 wells	4°C
IgA Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Human IgA Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

1. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. Prepare only as much reagent as is needed on the day of the experiment. When diluting the concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

1.1 1X Diluent N: Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

1.2 1X Wash Buffer: Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

1.3 1X Biotinylated IgA Detector Antibody:

1.3.1 The stock Biotinylated IgA Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated IgA Antibody for use in the assay. Observe the label for the "X" concentration on the vial of Biotinylated IgA Antibody.

1.3.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated IgA Antibody to prepare a 1X Biotinylated IgA Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Detector Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Δ Note Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated IgA Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated IgA Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated IgA Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated IgA Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated IgA Antibody to prepare 1X Biotinylated Antibody solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells: $\mathbf{(C_F / C_S) \times V_T = V_A}$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated IgA Antibody:

$$\mathbf{V_T - V_A = V_D}$$

First spin the Biotinylated IgA Antibody vial to collect the contents at the bottom.

1.3.3 Add calculated amount V_A of stock Biotinylated IgA Antibody to the calculated amount V_D of 1X Assay Diluent N. Mix gently and thoroughly.

1.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Δ Note Any remaining solution should be frozen at -20°C.

2. Standard Preparation

Always prepare a fresh set of standards for every use. Any remaining standard should be stored at -20°C after reconstitution and used within 30 days. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

2.1 Reconstitute the IgA Standard vial to generate a 100 ng/mL **Stock Standard**.

2.1.1 First consult the IgA Standard vial to determine the mass of protein in the vial.

2.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the IgA Standard vial to produce a 100 ng/mL IgA Stock Standard by using the equation:

C_S = Starting mass of IgA Standard (see vial label) (ng)

C_F = The 100 ng/mL IgA **Stock Standard** final required concentration

V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension: $\mathbf{(C_S / C_F) \times 1{,}000 = V_D}$

2.1.3 Briefly centrifuge the IgA Standard Vials to collect contents at the bottom of tube.

2.1.4 Reconstitute the IgA Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 100 ng/mL IgA **Stock Standard.** Mix gently and thoroughly.

2.2 Allow the reconstituted 100 ng/mL IgA **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

2.3 Label eight tubes #1 – 8.

2.4 Prepare the 50 ng/ml **Standard #1** by adding 120 µL of the reconstituted 100 ng/mL IgA **Stock Standard** to 120 µL of 1X Diluent N.

2.5 Add 120 µL of 1X Diluent N to tube #2 – 8.

2.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

2.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

2.8 Using the table below as a guide, prepare subsequent serial dilutions.

2.9 1X Diluent N serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Human IgA (ng/mL)
1	Step 2.4		50
2	120 µL Standard #1	120	25
3	120 µL Standard #2	120	12.5
4	120 µL Standard #3	120	6.25
5	120 µL Standard #4	120	3.130
6	120 µL Standard #5	120	1.560
7	120 µL Standard #6	120	0.781
8 (Blank)	N/A	120	0

3. Sample Preparation

Avoid repeated freeze-thaw cycles for all.

3.1 Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute urine 1:20 with 1X Diluent N and assay. If necessary, dilute samples within the range of 1:10 to 1:100. Depending on application needs, user should determine proper dilutions. Store samples at -20°C or below for up to 3 months.

3.2 Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute saliva 1:2,000 with 1X Diluent N and assay. If necessary, dilute samples within the range of 1:1,000 to 1:10,000. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at Store-20°C or below for up to 3 months.

3.3 Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute milk 1:10,000 with 1X Diluent N and assay. If necessary, dilute samples within the range of 1:2,000 to 1:40,000. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months.

3.4 Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes and collect plasma. A 160000-fold sample dilution is suggested into 1X Diluent N or within the range of 1:20,000 to 1:200,000. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant).

3.5 Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. A 160000-fold sample dilution is suggested into 1X Diluent N or within the range of 1:20,000 to 1:200,000. Depending on application needs, user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months.

3.6 Cerebrospinal Fluid (CSF): Collect cerebrospinal fluid (CSF) using a sample pot. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:500 into 1X Diluent N or within range of 1:200 to 1:2000, and assay. Depending on application needs, user should determine dilutions. The undiluted samples can be stored at -80°C or below for up to 3 months.

3.7 Cell Culture Supernatants: Centrifuge cell culture media at 1500 x g for 10 minutes to remove debris. Collect supernatants and assay. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Guidelines for Dilutions of 100-fold or Greater *(for reference only)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

4.1 Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature prior to use.
– We recommend that you assay all standards, controls and samples in duplicate.

4.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

4.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

4.3 Add 50 µL of IgA Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

4.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine, wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

4.5 Add 50 µL of 1X Biotinylated IgA Antibody to each well and incubate for one hour.

4.6 Wash microplate as described above.

4.7 Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

4.8 Wash microplate as described above.

4.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 12 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

4.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

4.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

4.12 Analyze the data as described below.

4.12.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

4.12.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.

4.12.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

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