Human IgG AccuSignal™ ELISA Kit – KAA9102

Rockland's human IgG AccuSignal™ ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay designed for the quantitative detection of human IgG in serum, plasma, and hybridoma cell supernatants.

I. KIT COMPONENTS

Human IgG AccuSignal™ ELISA Kit	
Catalog Number	**Component**
KAR001	Coating Buffer
KAR106-02	Capture Antibody
KAR002	Wash Buffer (10X)
KAR003	Assay Buffer (10x) to dilute into: Blocking buffer and Standard Reagent buffer
KAR005-02	Human IgG Control Standard
KAR607-02	Biotinylated Detection Antibody
KAR008	Streptavidin-Peroxidase Conjugate
KAR011	Streptavidin-Peroxidase Stabilizer
N/A	Empty Amber Vial
KAR009	Substrate Solution
KAR010	Stop Solution

II. STORAGE CONDITIONS

Store coating buffer, wash buffer, assay buffer and standard and reagent diluent at 4 ˚C for 3-6 months. Aliquot capture antibody and target protein standard contents and freeze at -20 ˚C. Store detection antibody and Streptavidin-Peroxidase conjugate vial at 4 °C prior to restoration. For extended storage aliquot contents and freeze at -20 °C or below. Avoid multiple freeze-thaw cycles. Store substrate solution at 4°C prior to opening. Protect from moisture and light.

III. INTRODUCTION

Human IgG AccuSignal™ ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. This assay employs a capture antibody specific for human IgG that can be coated onto 96-well plate. Standards and test samples are then pipetted into the wells. After sample binding, unbound proteins and molecules are washed off, and a biotinylated detection anti-human IgG antibody is added to the wells. The wells are washed with wash buffer. After washing away unbound biotinylated antibody, Streptavidin Peroxidase Conjugate is added to the wells and unbound conjugates are washed away. Substrate solution is added to the wells to visualize HRP enzymatic reaction. The reaction produces a blue color product, which turns yellow when the reaction is terminated by the addition of stop solution. The absorbance of the yellow product at 450 nm is proportional to the amount of Human IgG present in a sample.

IV. REQUIRED EQUIPMENT AND REAGENTS

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

- 96 well ELISA plate (a variety of ELISA plates can be used, e.g. Nunc MaxiSorp Plates)
- Microplate reader capable of measuring absorbance at 450nm
- Automated plate washer or a wash bottle
- Adjustable pipettes and pipette tips
- Clean tubes and Eppendorf tubes
- Distilled or deionized water
- Absorbent paper

V. GENERAL CONSIDERATIONS

Please read the following instructions before starting the experiment. Modifications to the kit components or procedures may result in the loss of performance.

- All ELISA reagents must be at room temperature before use.
- Before using the kit, briefly spin down the vials.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Do not allow 96-well plate to dry, this will inactivate components on plate.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure plates are properly sealed or covered during incubation steps.
- Do not mix or substitute reagents or materials from other kit lots or vendors.
- Do not vortex the standard during dilution, as this will destabilize the protein.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Do not contaminate the substrate solution. If the solution is blue before use, DO NOT USE IT.
- Include a standard curve each time the assay is performed.
- For statistical reasons, we recommend both standard and samples should be assayed with a minimum of two replicates (duplicates).
- Samples generating values greater than the highest standard should be further diluted in the appropriate sample dilution buffer.

VI. SANDWICH ELISA PROTOCOL

A. Reagent Preparation

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

Blocking Buffer

Dilute 30 mL of 10X assay buffer concentrate into 70 mL deionized or distilled water to yield 100 mL of blocking buffer.

1X Wash Buffer

If the 10X wash buffer contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 100 mL of 10X wash buffer into 900 mL deionized or distilled water to yield 1000 mL of 1X wash buffer.

1X Standard and Reagent Diluent

Dilute 20 mL of 10X assay buffer into 180 mL deionized or distilled water to yield 200 mL of 1X standard and reagent diluent buffer.

Preparation of 1 µg/mL capture antibody

Briefly spin the anti-human IgG capture antibody vial before use. Prepare capture antibody working dilution (1 µg/mL) in coating buffer, pH 9.5. For example, dilute 12 µL of 1 mg/mL stock capture antibody in 11.88 mL coating buffer.

The capture antibody stock (1 mg/mL) can be aliquoted and stored at -20 °C.

Preparation of 0.75 µg/mL biotinylated detection antibody

Gently knock down any visible powder on the walls of the vial before use. Reconstitute biotinylated antibody in 100 µL of 1X standard and reagent diluent (final concentration will be 1 mg/mL). Briefly spin streptavidin-peroxidase conjugate vial before use to ensure complete recovery.

Prepare biotinylated antibody working dilution (0.75 µg/mL) in 1X standard and reagent diluent. For example, dilute 9 µL of 1 mg/mL biotinylated antibody in 11.91 mL of 1X standard and reagent diluent.

The biotinylated antibody stock concentrate (1 mg/mL) can be aliquoted and stored at -20 °C.

Preparation of 0.1 µg/mL streptavidin-peroxidase conjugate solution

Gently knock down any visible powder on the walls of the vial before use. Reconstitute streptavidin peroxidase conjugate in 100 µL of stabilizer solution (final concentration will be 1 mg/mL). Briefly spin streptavidin-peroxidase conjugate vial before use to ensure complete recovery.

Using the empty amber vial provided, prepare streptavidin-peroxidase conjugate stock concentrate (10 µg/mL) in stabilizer. For example, dilute 8 µL of 1 mg/mL streptavidin-peroxidase conjugate in 792 µL stabilizer solution. The streptavidin-peroxidase conjugate stock concentrate can be aliquoted and stored at 4 °C (DO NOT FREEZE).

Prepare streptavidin-peroxidase conjugate working dilution (0.1 µg/mL) in 1X standard and reagent diluent. For example, dilute 120 µl of 10 µg/mL streptavidin-peroxidase conjugate in 11.88 mL of 1X standard and reagent diluent.

B. Standard Preparation

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

Preparation of 1 µg/mL standard

Dilute 2.5 µL of target protein standard (100 µg/mL) in 247.5 µL of 1X standard and reagent diluent.

Preparation of 100 ng/mL stock standard

Dilute 25 µL of 1 µg/mL target protein stock standard in 225 µL of 1X standard and reagent diluent.

Preparation of 12 ng/mL stock standard

Dilute 60 µL of 100 ng/mL target stock protein standard in 440 µL of 1X standard and reagent diluent.

- Prepare **Standard #1** by adding 250 µL of the 12 ng/mL **Stock Standard**, to 250 µL of 1X standard and reagent diluent. Mix thoroughly and gently.
- Pipette 250 µL of 1X standard and reagent diluent into remaining tubes.
- Prepare **Standard #2** by adding 250 µL Standard #1 to tube #2 and mix thoroughly.
- Prepare **Standard #3** by adding 250 µL Standard #2 to tube #3 and mix thoroughly.
- Using the table **below** as a guide, prepare further serial dilutions.
- 1X standard and reagent diluent serves as the zero (BLANK) (0 ng/mL).

Standard Dilution Preparation					
Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Concentration (ng/mL)	Final Concentration (ng/mL)
1	250	250	500	12.0	6.0
2	250	250	500	6.0	3.0
3	250	250	500	3.0	1.5
4	250	250	500	1.5	0.75
5	250	250	500	0.75	0.375
6	250	250	500	0.375	0.187
7	250	250	500	0.187	0.093
8	0	250	250	0	0

C. Sample Preparation

1X standard and reagent diluent should be used for dilution of serum and plasma.

The levels of the target protein may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Suggested dilution for normal serum/plasma: 10,000,000-fold.

D. Assay Procedure

Samples and reagents should be mixed thoroughly and evenly after dilution. A standard curve for human IgG detection should be prepared for each experiment.

For statistical reasons, it is recommended to assay all standards, controls and samples in duplicate.

1. Dilute the capture antibody to a final concentration of 1 µg/mL in coating buffer as described above.
2. Add 100 µL of capture antibody into appropriate wells. Cover wells and incubate overnight at room temperature.
3. Remove the cover and discard the capture antibody solution. Invert the plate and blot it against clean paper towels or other absorbent material. Do NOT let the wells dry completely at any time.
4. Add 250 µL of blocking buffer to each well and incubate at room temperature for 1-3 hours.

5. Discard the blocking solution and add 100 µL of each standard (see Standard Preparations above) and sample into appropriate wells. Cover wells and incubate for 1 hour at room temperature.
6. Discard the solution and wash the plate 4 times with 1X wash buffer. Wash by filling each well with 300 µL of wash buffer using a multi-channel pipette or auto washer. Complete removal of liquid at each step is important to good performance. (Note: for automated washing, aspirate all wells and wash four times with 1X wash buffer, overfilling the wells with wash buffer). After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels or other absorbent material.
7. Add 100 µL of biotinylated anti-Human IgG detection antibody working solution to each well, seal the plate and incubate for 1 hour at room temperature.
8. Discard the solution and repeat the wash as described in step 6.
9. Add 100 µL of streptavidin-peroxidase conjugate working solution (see preparation of streptavidin-peroxidase conjugate solution above) to each well. Incubate for 30 minutes at room temperature.
10. Discard the solution. Repeat the wash as described in step 6.
11. Add 100 µL of substrate solution to each well. Seal the plate and incubate for 30 min at room temperature in the dark. (Note: the optimal incubation time should be determined by the end user).
12. Add 100 µL of stop solution to each well.
13. Read at 450 nm in a microplate reader immediately.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	Blk	Blk	8	8	16	16	24	24	32	32	40	40

CALCULATIONS

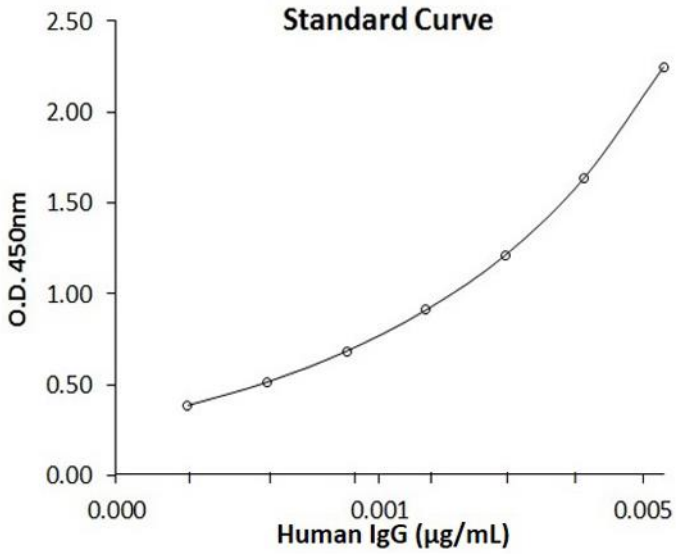
For calculations, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human IgG concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the concentrations obtained from interpolation by the dilution factor.

VII. EXAMPLE DATA OBTAINED FROM HUMAN IgG

Concentration (ng/mL)	0	0.094	0.188	0.375	0.75	1.5	3.0	6.0
O.D	0	0.39	0.52	0.69	0.91	1.21	1.64	2.25

VIII. HUMAN IgG ELISA KIT STANDARD CURVE EXAMPLE



Range	0.094 ng/mL-6.0 ng/mL
Sensitivity	<100 pg/mL
Specificity	Natural and recombinant Human IgG
Recovery	100%

ASSAY SUMMARY

- Prepare all reagents, samples and standards as instructed.
- Add capture antibody to each well used. Incubate the plate overnight at room temperature.
- Add prepared blocking buffer to each well. Incubate for 1-3 hours at room temperature.
- Add standards or samples to each well used. Incubate the plate for 1 hour at room temperature. Wash plate four times with wash buffer.
- Add prepared biotinylated antibody to each well. Incubate the plate for 1 hour at room temperature. Wash plate four times with wash buffer.
- Add streptavidin-peroxidase conjugate working solution. Incubate the plate for 30 min at room temperature. Wash plate four times with wash buffer.
- Add substrate solution to each well. Incubate the plate in dark for 30 min or until color develops.
- Add stop solution. Read at 450nm.

IX. TROUBLESHOOTING

Weak or No Signal

Possible Cause	Solution
Problem with the standard	Use new sample Check that the standard is appropriately handled
Incubation time too short	Follow the exact guidelines for incubation time (If the problem persists, try incubating samples at 4°C overnight)
Incubation temperature too low	Ensure incubations are done at correct temperature Before proceeding
Incompatible sample type	Use sample that the assay is known to detect as a positive control (Include such control in your experiment)
Target present below detection limit	Decrease dilution factor or concentrate samples
Incorrect/Insufficient/No substrate	Check the substrate identity Increase concentration or amount of substrate
Antibody stored at 4°C for several weeks or subjected to repeat freeze-thaw cycles	Use fresh aliquot of antibody that has been stored at -20°C or below
Incorrect reagents added/ prepared; Missing reagents	Check protocol, ensure correct reagents are added in proper order and prepared to correct concentrations
Expired/Contaminated reagents	Prepare fresh/uncontaminated reagents
Incorrect storage of components	Check storage conditions for the kit (Kit need to be stored at 4°C)
Excessive plate washing	Gently pipette wash buffer (manual method) Ensure correct pressure (automatic wash system)
Wells dry out	Cover plate using adhesive cover at all incubation times
Plate read at incorrect detection wavelength	Use recommended wavelength/filter Ensure plate reader is set correctly for substrate used

Slow color development	Prepare substrate immediately before use Allow longer incubation time Ensure stock solution is unexpired and uncontaminated

Saturated Signal

Possible Cause	Solution
High sample concentration	Use higher sample dilutions (Determine the optimal dilutions by titration assay)
Substrate color changed before use	Prepare substrate immediately before use
Incubation time too long	Follow the exact guidelines for incubation times
Excessive antibody	Repeat the assay with lower antibody concentrations to find the optimal one for your experiment
Contaminated buffers or Peroxidase	Prepare and use fresh buffers
Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and dry the plate on a paper towel
Plate adhesive cover not used or re-used	During incubations, cover plates with adhesive cover. Use a fresh cover every time the used cover is removed from the plate
Plate read at incorrect wavelength	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used
Excess time before plate reading	Read your plate within 30 minutes after adding the stop solution

High Background

Possible Cause	Solution
Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and pat dry the plate on a paper towel
Excessive antibody	Repeat the assay with lower antibody concentrations to find the optimal one for your experiment
Cross reactivity	Run appropriate controls
Incubation temperature too high	Optimize incubation temperature for your assay (antibodies bind optimally at very specific temperature)
Reagents were not mixed properly	Thoroughly mix all reagents and samples before pipetting solutions into wells
Blanks contaminated with samples	Change pipette tips when switching between blanks and samples
Sample contaminated with enzymes	Test samples with substrate alone to check for contaminating enzymes
Contaminated substrate solution	Use a clean container to check that the substrate in not contaminated (TMBE substrate should be clear and colorless before adding to wells)
Substrate exposed to light	Carry out substrate incubation in dark

Evaporation of solution from well during incubation	Always incubate with a cover on the plate
Incubation time too long	Follow the exact guidelines for incubation times
Incorrect standard curve dilutions	Check pipetting techniques Check calculations
Unstopped color development	Use Stop solution to prevent over-development
Excessive time lapsed before plate reading	Read your plate within 30 minutes after adding the substrate
Incorrect plate reading setting	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used

Low Sensitivity

Possible Cause	Solution
Improper storage of ELISA kit	Store all reagents as recommended
Insufficient target	Reduce sample dilution or concentrate sample
Inactive substrate	Ensure reporter enzyme has the expected activity
Incompatible sample type	Include positive control in your experiment
Interfering ingredients in buffers and sample	Check reagents for any interfering chemicals, e.g. sodium azide in antibodies inhibit HRP enzyme; EDTA used as anticoagulant for plasma collection inhibits enzymatic reactions
Mixing or substituting reagents from different kits	Avoid mixing components from different kits
Incorrect plate reading setting	Use recommended wavelength/filter Ensure plate reader is set correctly for the substrate used

Poor Standard Curve Generation

Possible Cause	Solution
Improper standard solution	Confirm dilutions are done correctly Prepare new standard curve as appropriate
Standard improperly reconstituted	Briefly spin vial before opening Inspect for undissolved material after reconstituting
Standard degraded	Store and handle standard as recommended
Pipetting error	Use calibrated pipettes and proper pipetting technique
Insufficient washing	Follow the exact guidelines At the end of each washing step, flick the plate over a sink and pat dry the plate on a paper towel
Poorly mixed reagents	Thoroughly mix reagents
Plates stacked during incubation	Keep plates separated if not using rotating plates

Poor Replicate Data

Possible Cause	Solution
Bubbles in wells	Ensure no bubbles are present prior to reading the plate
Insufficient washing of wells	Carefully wash wells Check that all ports of the plate washer are unobstructed
Incomplete reagent mixing	Ensure all reagents are mixed thoroughly
Inconsistent pipetting	Use calibrated pipettes and proper pipetting techniques Use a new cover every time the used cover is removed from the plate
Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage (e.g. minimize freeze/thaw cycles)
Particulates in samples	Remove the particulates by centrifugation
Cross-well contamination	Ensure plate covers and pipette tips are not contaminated with reagents
Edge effect (higher or lower OD in peripheral wells than in central wells)	Ensure plates and reagents are kept at temperatures as instructed During incubation, seal the plate completely and avoid stacking plates

Inconsistent Assay-to-Assay Results

Possible Cause	Solution
Insufficient washing of wells	Carefully wash wells Check that all ports of the plate washer are unobstructed
Varied incubation temperatures	Adhere to recommended incubation temperature
Variation in protocol	Adhere to the same protocol from experiment to experiment
Plate cover not used or reused	During incubations, cover plates with plate cover Use a new cover every time the used one is removed
Incorrect dilutions	Confirm dilutions are done correctly for standard solutions Prepare new standard curve as appropriate
Contaminated buffers	Prepare and use fresh buffers

Slow Color Development

Possible Cause	Solution
Substrates too old, contaminated or used at incorrect pH	Prepare fresh substrates at correct pH
Expired/Contaminated solutions	Prepare fresh reagents before use
Incorrect incubation temperature	Ensure plates and reagents are kept at temperatures as instructed During incubation, seal the plate completely and avoid stacking plates

X. RELATED PRODUCTS

Component	Catalog #	Size
10x TBS pH 7.5	MB-012	1000 mL
10x TTBS pH 7.5	MB-013	1000 mL
10X PBS pH 7.2	MB-008	1000 mL
10x PBST pH 7.2	MB-075-1000	1000 mL
10xPBS Fish gel concentrate	MB-066-1000	10 Pack