ab108854

Human Alpha 1 Acid Glycoprotein / AGP ELISA Kit

Instructions for Use

For the quantitative measurement of Human Alpha 1 Acid Glycoprotein / AGP concentrations in plasma and serum.

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

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1. Introduction

Alpha 1 Acid Glycoprotein / AGP is an acute-phase protein secreted by the liver which under conditions of inflammation increase several-fold in concentration. An elevated serum level of acute-phase inflammatory markers is associated with an increased risk of cardiovascular disease Urinary orosomucoid excretion rate predicts cardiovascular mortality in patients with Type II diabetes. Alpha 1 Acid Glycoprotein / AGP can be used as a marker for inflammation, chronic alcohol drinking, chronic kidney disease, and asthma.

ab108854 employs a quantitative competitive enzyme immunoassay technique that measures Human plasma and serum Alpha 1 Acid Glycoprotein / AGP in less than 3 hours. A polyclonal antibody specific for Human Alpha 1 Acid Glycoprotein / AGP has been pre-coated onto a 96-well microplate with removable strips. Alpha 1 Acid Glycoprotein / AGP in standards and samples is competed by a biotinylated Alpha 1 Acid Glycoprotein / AGP sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 25 µL standard or sample to each well and immediately add 25 µL prepared Human Alpha 1 Acid Glycoprotein / AGP to each well.

Incubate 2 hour at room temperature.

Wash the microplate 5 x with 1X Wash Buffer.

Add 50 µL of Streptavidin-Peroxidase Conjugate.

Incubate 30 minutes at room temperature.

Wash the microplate 5 x with 1X Wash Buffer.

Add 50 µL of Chromogen Substrate to each well.

Incubate 15 minutes or till the optimal blue colour density develops.

Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

3. Kit Contents

- Alpha 1 Acid Glycoprotein / AGP Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Alpha 1 Acid Glycoprotein / AGP.
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Human Alpha 1 Acid Glycoprotein / AGP Standard: Human Alpha 1 Acid Glycoprotein / AGP in a buffered protein base (lyophilized).
- 1X Biotinylated Human Alpha 1 Acid Glycoprotein / AGP (lyophilized)
- 10X Diluent N Concentrate: A 10-fold concentrated buffered protein base (30 mL).
- 20X Wash Buffer Concentrate: A 20-fold concentrated buffered surfactant (30 mL).
- 100X Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µL)
- Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (7 mL).
- Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (11 mL).

4. Storage and Handling

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, 10X Diluent N Concentrate, 20X Wash Buffer Concentrate, Stop Solution, and Chromogen Substrate at 2-8°C.
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- 1X Diluent N may be stored for up to 1 month at 2-8°C
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with 1X Diluent N and at -20°C after reconstituting with 1X Diluent N.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 450nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes and multiple channel.
- Distilled or deionized reagent grade water.

6. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

7. Technical Hints

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.
- The coefficient of determination of the standard curve should be ≥0.95 and the highest O.D. should be more than 1.0.
- Cover or cap all kit components and store at 2-8° C when not in use.
- Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.

- Samples should be collected in pyrogen/endotoxin-free tubes.
- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Do not mix or interchange different reagent lots from various kit lots.
- Read absorbance immediately after adding the stop solution.
- Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.

8. Preparation of Reagents

Sample Collection:

1. **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and assay. A 1000-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. 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 anticoagulant.)
2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. A 1000-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

Freshly dilute all reagents and bring all reagents to room temperature before use. When diluting 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. **1X Diluent N:** Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 to 8°C.

2. **Standard Curve:** Reconstitute the Alpha 1 Acid Glycoprotein / AGP Standard with the appropriate amount of 1X Diluent N to generate a solution of 4 µg/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (4 µg/mL) 1:2 with 1X Diluent N to produce 2, 1, 0.5, 0.25, and 0.125 µg/mL solutions. 1X Diluent N serves as the zero standard (0 µg/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Alpha 1 Acid Glycoprotein / AGP] (µg/mL)
P1	1 Part Standard (4 µg/mL)	4.000
P2	1 part P1 + 1 part 1X Diluent N	2.000
P3	1 part P2 + 1 part 1X Diluent N	1.000
P4	1 part P3 + 1 part 1X Diluent N	0.500
P5	1 part P4 + 1 part 1X Diluent N	0.250
P6	1 part P5 + 1 part 1X Diluent N	0.125
P8	1X Diluent N	0.000

3. **1X Biotinylated Human Alpha 1 Acid Glycoprotein / AGP:** Reconstitute Biotinylated Human Alpha 1 Acid Glycoprotein / AGP with 4 mL 1X Diluent N to produce a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Any

remaining solution should be frozen at -20°C and used within 30 days.

4. **1X Wash Buffer:** Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water.
5. **1X SP Conjugate:** Spin down the 100X SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N. Any remaining solution should be frozen at -20°C.

9. Assay Method

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
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 dessicator.
3. Add 25 µL of Human Alpha 1 Acid Glycoprotein / AGP Standard or sample per well and immediately add 25 µl of Biotinylated Human Alpha 1 Acid Glycoprotein / AGP to each well (on top of the Standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
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.

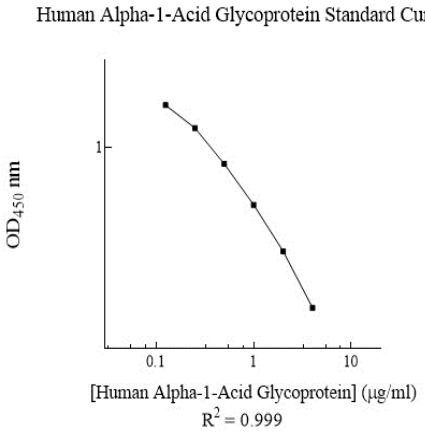
5. Add 50 µl of Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash microplate as described above.
7. Add 50 µl of Chromogen Substrate to each well and incubate in ambient light for about 20 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
8. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
9. 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.

10. Data Analysis

Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

A. Typical Data

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



B. Sensitivity

The minimum detectable dose of Alpha 1 Acid Glycoprotein / AGP is typically 94 ng/mL.

C. Recovery

Standard Added Value: 0.25 – 2 µg/mL

Recovery %: 88 – 112

Average Recovery %: 98

D. Reproducibility

Intra-Assay: CV = 5.3%

Inter-Assay: CV = 11.1%

E. Linearity

Sample Dilution	Average % of Expected Value	
	Plasma	Serum
1:500	89	98
1:1000	109	99
1:2000	110	103

11. Specificity

Species	% Cross Reactivity
Canine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Bovine	None
Rabbit	None
Equine	None
Species	**% Cross Reactivity**
alpha-1-Acid Glycoprotein 2	3%

12. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:

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