# Human alpha-1-Acid Glycoprotein ELISA Kit

**Vertrieb:**
**L O X O GmbH** Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
**Telefon** +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
**E-Mail:** info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

**Hinweis/Note:**
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

## Assay Summary

Add 50 µl of standard/samples per well.
Incubate 2 hours.

Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.

Wash, then add 50 µl of SP per well.
Incubate 30 minutes.

Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 12 minutes.

Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

# Assay Template

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |
| 4 | | | | | | | | |
| 5 | | | | | | | | |
| 6 | | | | | | | | |
| 7 | | | | | | | | |
| 8 | | | | | | | | |
| 9 | | | | | | | | |
| 10 | | | | | | | | |
| 11 | | | | | | | | |
| 12 | | | | | | | | |

# AssayMax Human alpha-1-Acid Glycoprotein ELISA Kit

Catalog No. EG5101-1
Sample Insert/Reference Only

## Introduction

Alpha-1-acid glycoprotein (AGP) or orosomucoid is an acute-phase plasma glycoprotein. It is synthesized in the liver and secreted into the plasma. The protein is a single polypeptide chain of 183 amino acids containing high carbohydrate content (45%) of its 41 kDa molecular weight (1). As a consequence of acute infections or inflammation, the plasma concentration of AGP increases considerably. The elevated serum level of AGP is associated with an increased risk of cardiovascular disease. Urinary AGP excretion rate predicts cardiovascular mortality in patients with Type II diabetes (2). AGP can be used as a marker for acute inflammation (3), chronic alcohol drinking (4), chronic kidney disease (5), and asthma (6).

## Principle of the Assay

The AssayMax Human alpha-1-Acid Glycoprotein ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative sandwich enzyme immunoassay technique that measures cell culture supernatant, saliva, milk, and urine AGP in less than 4 hours. A polyclonal antibody specific for AGP has been pre-coated onto a microplate. AGP in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for AGP, which is recognized by a 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.

## Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**

- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

## Reagents

- **Human AGP Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human AGP.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human AGP Standard:** Human AGP in a buffered protein base (200 ng, lyophilized).
- **Biotinylated Human AGP Antibody (30x):** A 30-fold biotinylated polyclonal antibody against human AGP (270 µl).
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

## Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and biotinylated antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C. after reconstituting with diluent.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.

- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

## Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Urine dilution is suggested at 1:50 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Saliva dilution is suggested at 1:50 in MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Milk dilution is suggested at 1:800 in MIX Diluent; however, the user should determine the optimal dilution factor. 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.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 200 ng of Human AGP Standard with 1 ml of MIX Diluent to generate a solution of 200 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (200 ng/ml) 1:2 with MIX Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

| Standard Point | Dilution | [AGP] (ng/ml) |
|---|---|---|
| P1 | Standard (200 ng/ml) | 200.0 |
| P2 | 1 part P1 + 1 part MIX Diluent | 100.0 |
| P3 | 1 part P2 + 1 part MIX Diluent | 50.00 |
| P4 | 1 part P3 + 1 part MIX Diluent | 25.00 |
| P5 | 1 part P4 + 1 part MIX Diluent | 12.50 |
| P6 | 1 part P5 + 1 part MIX Diluent | 6.250 |
| P7 | 1 part P6 + 1 part MIX Diluent | 3.125 |
| P8 | MIX Diluent | 0.000 |

- **Biotinylated Human AGP Antibody (30x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:30 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

## Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human AGP Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human AGP Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

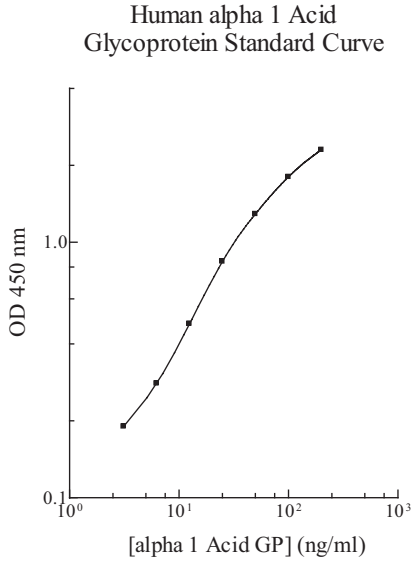
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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.

## 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.

## Standard Curve

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



## Performance Characteristics

- The minimum detectable dose of AGP is typically 3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.0% respectively.

## Linearity

| | Average Percentage of Expected Value | |
|---|---|---|
| **Sample Dilution** | **Urine** | **Saliva** |
| **1:25** | 89% | 91% |
| **1:50** | 99% | 98% |
| **1:100** | 104% | 101% |

| | Average Percentage of Expected Value |
|---|---|
| **Sample Dilution** | **Milk** |
| **1:400** | 94% |
| **1:800** | 100% |
| **1:1600** | 98% |

## Recovery

| | |
|---|---|
| **Standard Added Value** | 5 – 50 ng/ml |
| **Recovery %** | 83-115 % |
| **Average Recovery %** | 97% |

## Cross-Reactivity

| **Species** | **% Cross Reactivity** |
|---|---|
| Canine | None |
| Monkey | <5% |
| Mouse | None |
| Rat | None |
| Swine | None |
| Bovine | None |
| Rabbit | None |

## References

(1) Fournier T et al. (2000) Biochim Biophys Acta. 1482(1-2):157-171
(2) Christiansen MS et al. (2002) Diabetologia 45(1): 115-120
(3) Magid E et al. (2005) Clinical Chemistry 51(11): 2052-2058
(4) Tsutsumi M et al. (2001) Alcohol. 25(3): 181-184
(5) Romao JE Jr et al. (2006) Am J Nephrol. 26(1): 59-66
(6) Van Den Heuvel MM et al. (2000) Am J Respir Crit Care Med. 161(6):1972-1978

Version 1.3

## Related Products

- EG5001-1 AssayMax Human Alpha1-Acid Glycoprotein ELISA Kit (Plasma and Serum Samples)