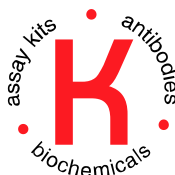


KAMIYA BIOMEDICAL COMPANY

Monkey Alpha-1 Acid Glycoprotein ELISA

For the quantitative determination of alpha-1 acid glycoprotein in monkey serum or plasma.

Cat. No. KT-490

For Research Use Only.

PRODUCT INFORMATION

Monkey Alpha-1 Acid Glycoprotein ELISA
Cat. No. KT-490

PRODUCT
The ***K-ASSAY*®** Monkey Alpha-1 Acid Glycoprotein ELISA is an enzyme immunoassay for the quantitative determination of alpha-1 acid glycoprotein (AGP) in monkey serum or plasma. For research use only.

INTRODUCTION
AGP is an acute phase protein that is elevated in serum due to injury, infection or disease. Our studies have demonstrated a 5 to 10-fold increase of AGP in serum of monkeys with on-going infection.

PRINCIPLE
The assay uses affinity purified monkey AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated monkey AGP antibodies for detection. Calibrators and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in AGP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If AGP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of AGP is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- AGP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- AGP calibrator (lyophilized)
- 20x Wash solution, 50 mL
- 10x Diluent, 25 mL
- TMB, 11 mL
- Stop solution, 11 mL

Due to international import/export restrictions of monkey derived products, the AGP calibrator supplied with this kit is of non-monkey origin. The calibration curve obtained with this material is identical to that obtained with monkey AGP.

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Curve fitting software

GENERAL INSTRUCTIONS
1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10X stock with nine volumes of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. The AGP calibrator is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved **(the reconstituted calibrator remains stable for at least 10 days at 4°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended)**.
2. Label 6 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25 and 3.13 ng/mL.
3. In the tube labeled 100 ng/mL prepare the 100 ng/mL calibrator by mixing 153.7 µL of reconstituted calibrator with 346.3 µL of diluent.
4. Dispense 250 µL of diluent into the tubes labeled 50, 25, 12.5, 6.25 and 3.13 ng/mL.
5. Prepare the 50 ng/mL calibrator by mixing 250 µL of the 100 ng/mL calibrator with 250 µL of diluent in the tube labeled 50 ng/mL.
6. Similarly prepare the remaining calibrators by two-fold serial dilution.

SAMPLE PREPARATION

We found that AGP is present in monkey serum at concentrations of 0.1 to 2 mg/mL. To obtain values within the range of the calibration curve we suggest that samples initially be diluted 20,000-fold using the following procedure for each sample to be tested:

1. Dispense 198 µL and 497.5 µL of 1x diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of diluent. This provides a 100-fold diluted sample.
3. Mix 2.5 µL of the 100-fold diluted sample with the 497.5 µL of diluent in the second tube. This provides a 20,000-fold dilution of the sample.

AGP levels may vary with animal husbandry and study protocols. Therefore, please be aware that optimal serum or plasma dilutions should be determined empirically

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1X wash solution using a plate washer (400 µL/well).
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µL of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

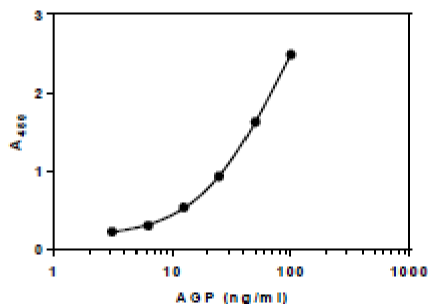
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and calibration curve in each experiment.

AGP (ng/mL)	Absorbance (450 nm)
100	2.486
50	1.626
25	0.932
12.5	0.533
6.25	0.302
3.13	0.224



STORAGE

The unused kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date.

FOR RESEARCH USE ONLY

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