Datasheet for ABIN956166

ORM1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	ORM1
Reactivity:	Monkey
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The Monkey -1-AGP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-monkey -1-AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-monkey -1-AGP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in -1-AGP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of -1-AGP is proportional to the optical density of the test sample.

Product Details

Components:	Anti-monkey -1-AGP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8) Enzyme Conjugate Reagent, 11 mL Monkey -1-AGP Calibrator (lyophilized) Note: International import/export restrictions apply to monkey derived products. In order to avoid such restrictions the monkey -1-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 -1-AGP. Diluent (10X), 25 mL Wash Solution (20X), 50 mL TMB Reagent (One-Step), 11 mL Stop Solution (1N HCl), 11 mL.
Material not included:	Precision pipettes and tips Distilled or de-ionized water Polypropylene or glass tubes Vortex mixer Absorbent paper or paper towels Micro-Plate incubator/shaker mixing speed of ~150 rpm A microtiter plate reader capable of measuring absorbance at 450 nm, with a bandwidth of 10 nm or less and an OD range of 0-4 OD Graph paper (PC graphing software is optional)

Target Details

Target:	ORM1
Alternative Name:	Alpha-1 Acid Glycoprotein (ORM1 Products)
Background:	Alpha-1-AGP is an acute phase serum protein. Studies have demonstrated that levels of alpha-1-AGP are elevated five to ten fold in serum of monkeys undergoing veterinary treatment. alpha-1-AGP is a useful biomarker of tissue injury, inflammation and infection in monkeys.
Pathways:	Response to Growth Hormone Stimulus

Application Details

Plate:	Pre-coated
Sample Preparation:	General Note: Our studies find that -1-AGP may be present in monkey serum at concentrations of 0.2 to 2 mg/mL. In order 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 two tubes. 2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of 1X 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. 4. Repeat this procedure for each sample to be tested.
Assay Procedure:	1. Secure the desired number of coated wells in the holder. 2. Dispense 100 µL of calibrators and samples into the wells (we recommend that samples be tested in duplicate). 3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes. 4. Remove the incubation mixture using a plate washer or by flicking plate contents into an appropriate Bio-waste container. 5. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible. 6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets. 7. Add 100 µL of enzyme conjugate reagent into each well. 8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes. 9. Wash as detailed in 4 and 5 above. 10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets. 11. Dispense 100 µL of TMB Reagent into each well. 12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes. 13. Stop the reaction by adding 100 µL of Stop Solution to each well. 14. Gently mix. It is important to make sure that all the blue color changes to yellow. 15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
Calculation of Results:	1. Calculate the average absorbance values (A450) for each set of reference calibrators, and samples. 2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

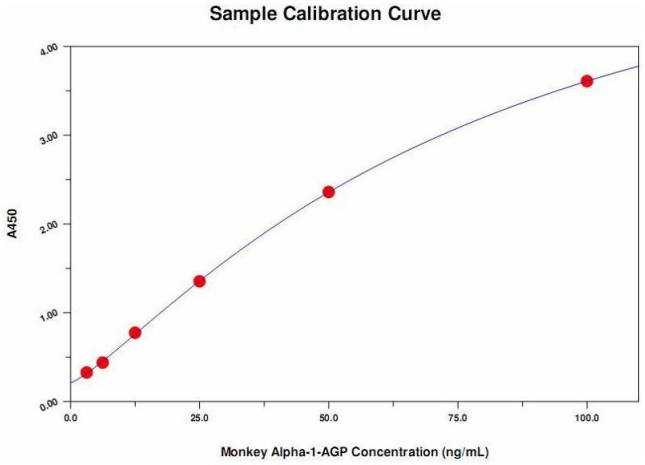
Application Details

	3. Using the mean absorbance value for each sample, determine the corresponding concentration of -1-AGP in ng/mL from the calibration curve. 4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of -1-AGP in the serum/plasma sample. 5. If available, PC graphing software may be used for the above steps. 6. If the OD450 values of samples fall outside the calibration curve when tested at the suggested dilution of 20,000, samples should be diluted appropriately and re-tested.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	The unused kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.
Expiry Date:	The expiry date is stated on the label.

Images



ELISA

Image 1.