Datasheet for ABIN956106

LOC100192394 ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	LOC100192394
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The Mouse -macroglobulin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-mouse -macroglobulin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-mouse -macroglobulin 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 -macroglobulin 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 absorbance is measured at 450 nm. The concentration of -macroglobulin is proportional to the optical density of the test sample.

Product Details

Components:	Anti-mouse -macroglobulin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8) Enzyme Conjugate Reagent, 11 mL Mouse -Macroglobulin Calibrator (lyophilized) Diluent, 30 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 Plate reader with an optical density range of 0-4 at 450 or 405 nm Graph paper (PC graphing software is optional)

Target Details

Target:	LOC100192394
Alternative Name:	alpha-macroglobulin (LOC100192394 Products)
Background:	Alpha-macroglobulin is a serum proteinase inhibitor that consists of two major (Mr 163,000 and 35,000) and one minor (Mr 185,000) polypeptide chains. It is a negative acute phase reactant, the levels of which decrease in mouse serum or plasma as a result of inflammation. It has also been demonstrated that mouse alpha-macroglobulin levels increase significantly with age, after gonadectomy and during pregnancy.

Application Details

Plate:	Pre-coated
Sample Preparation:	General Note: alpha-macroglobulin is present in normal mouse serum at a concentration of ~2.5 mg/mL. In order to obtain values within the range of the calibration curve, we suggest that samples be diluted 50,000 fold using the following procedure for each sample to be tested. 1. Dispense 998 µL of water and 297 µL of diluent into separate tubes. 2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 998 µL of diluent.

	This provides a 500 fold diluted sample. 3. Mix 3 µL of the 500 fold diluted sample with the 297 µL of diluent in the second tube. This provides a 50,000 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 (calibrators and samples should 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 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 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 to 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 5 minutes. Please Note: If the A450 of the high calibrator(s) exceeds the limits of the plate reader, absorbance of all wells may be determined at 405 nm instead.
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 concentration on the horizontal or X-axis. 3. Using the mean absorbance value for each sample, determine the corresponding concentration of -macroglobulin in ng/mL from the calibration curve.

Application Details

	4. Multiply the derived concentration by the dilution factor to determine the actual concentration of -macroglobulin in the serum/plasma sample. 5. 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 a dilution of 50,000, samples should be diluted appropriately and re-tested.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	The 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. The 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.