Datasheet for ABIN956374

ANG ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	ANG
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	78-5000 pg/mL
Minimum Detection Limit:	78 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of rat ANGPT1 in serum, plasma and other biological fluids.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of rat ANGPT1. No significant cross- reactivity or interference between rat ANGPT1 and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us - complete the cross-reactivity detection between rat ANGPT1 and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	The minimum detectable dose of rat ANGPT1 is typically less than 34 pg/mL. The Sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two

Product Details

	standard deviations - the mean optical density value of twenty zero calibrator replicates and calculating the corresponding concentration.
Characteristics:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to ANGPT1. Calibrators or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for ANGPT1. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain ANGPT1, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm +/- 10 nm. The concentration of ANGPT1 in the samples is then determined by comparing the O.D. of the samples to the calibration curve.
Components:	Pre-coated, ready to use 96-well strip plate (1x) Calibrator (lyophilized) (2x) Calibrator Diluent (1 x 20 mL) Detection Reagent A (1 x 120 µL) Detection Reagent B (1 x 120 µL) Assay Diluent A (2X concentrate) (1 x 6 mL) Assay Diluent B (2X concentrate) (1 x 6 mL) TMB Substrate (1 x 9 mL) Stop Solution (1 x 6 mL) Wash Buffer (30X concentrate) (1 x 20 mL) Plate sealer for 96 wells (4x).
Material not included:	1. Microplate reader with 450 +/- 10 nm filter. 2. Precision single or multi-channel pipettes and disposable tips. 3. Eppendorf Tubes for diluting samples. 4. Deionized or distilled water. 5. Absorbent paper for blotting the microtiter plate. 6. Container for Wash Solution

Target Details

Target:	ANG
Alternative Name:	ANG1 (ANG Products)

Application Details

Comment:	The calibration curve concentrations used for the ELISA's were 5, 000 pg/mL, 2, 500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL, 78 pg/mL.
Plate:	Pre-coated
Assay Procedure:	1. Determine wells for diluted calibrator, blank and sample. Prepare 7 wells for calibrator, 1 well for blank. Add 100 µL each of dilutions of calibrator (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37° C. 2. Remove the liquid of each well, don't wash. 3. Add 100 µL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37° C after covering it with the Plate sealer. 4. Aspirate the solution and wash with 350 µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. 5. Add 100 µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37° C after covering it with the Plate sealer. 6. Repeat the aspiration/wash process for total five times as conducted in step 4. 7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 25 minutes at 37° C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution. 8. Add 50 µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately. Note: 1. Assay preparation: Keep appropriate numbers of strips for 1 experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20°C. 2. Sample or reagent additions: Please use the freshly prepared Calibrator. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all calibrators and samples, although not required, is

Application Details

	recommended. To avoid cross- contamination, change pipette tips between additions of calibrators, samples, and reagents. Also, use separated reservoirs for each reagent. 3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled. 4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. 6. TMB Substrate is easily contaminated. Please protect it from light. 7. The environment humidity which is less than 60% might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.
Calculation of Results:	Average the duplicate readings for each calibrator, control, and samples and subtract the average zero calibrator optical density. Create a calibration curve on log-log graph paper, with ANGPT1 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the calibrator points and it can be determined by regression analysis. Using some plot software is also recommended. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	All the reagents should be kept according to the labels on vials. The Calibrator, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20° C upon being received. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above.
Expiry Date:	The expiry date is stated on the label.