Datasheet for ABIN956221

EGR1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	EGR1
Reactivity:	Mouse
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	This EGR-1 ELISA kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The coated well immunoenzymatic assay for the quantitative measurement of serum EGR-1 utilizes a monoclonal anti-EGR-1 and a EGR-1-HRP conjugate. The assay sample and buffer are incubated together with the anti-EGR-1 antibody coated plate for sixty and washed. The diluted EGR-1-HRP conjugate is then added to each well and incubated. After the incubation period, the wells are decanted and washed three times. The wells are then incubated with a substrate for the enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stopping solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the EGR-1 concentration since EGR-1 from samples and EGR-1-HRP conjugate compete for the anti-EGR-1 antibody binding site. Since the number of sites is limited, as more sites are occupied by EGR-1 from the sample,

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	fewer sites are left to bind EGR-1-HRP conjugate. Calibrators of known EGR- 1 concentrations are run concurrently with the samples being assayed and a calibration curve is plotted relating the intensity of the color (Optical Density) to the concentration of EGR-1. The unknown EGR-1 concentration in each sample is interpolated from this curve.
Components:	Microtiter Plate: 96 wells Calibrator 1 (0 ng/mL) Calibrator 2 (0.5 ng/mL) Calibrator 3 (1.0 ng/mL) Calibrator 4 (2.5 ng/mL) Calibrator 5 (5.0 ng/mL) Calibrator 6 (10 ng/mL) Enzyme Conjugate (1 x 6 mL) Substrate A (1 x 6 mL) Substrate B (1 x 6 mL) Stop Solution (1 x 6 mL) Wash Buffer (100X concentrate) (1 x 6 mL) Lysis Buffer Solution (1 x 6 mL) Note: The lysis buffer solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate, If the sample is serum or blood plasma, then the lysis buffer solution is a superfluous reagent.
Material not included:	1. Microplate reader capable of measuring absorbance at 450 nm. 2. Precision pipettes to deliver 2 mL to 1 mL volumes. 3. Adjustable 10-100 mL pipettes for reagent preparation. 4. 100 mL and 1 liter graduated cylinders. 5. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi- channel pipette is desirable for large assays.) 6. 37°C incubator. 7. Absorbent paper. 8. Distilled or de-ionized water 9. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired. 10. Tubes to prepare calibrator or sample dilutions.

Target Details

Target:	EGR1

Target Details

Alternative Name:	Early Growth Response Protein 1 (EGR1) (EGR1 Products)
Pathways:	Regulation of Carbohydrate Metabolic Process, Regulation of long-term Neuronal Synaptic Plasticity

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Plate:	Pre-coated
Reagent Preparation:	Bring all kit components and samples to room temperature (18-25°C) before use. Dispense 5 µL of lysis buffer solution into 50 µL specimens, mix and stand for one hour (The proportion of lysis buffer and specimens shall be no less than 1:10). (NOTE: This step is required when the sample is cell culture fluid & body fluid & tissue homogenate, If the sample is serum or blood plasma, then this step should be skipped.) Wash Solution Dilute 10 mL of Wash Solution concentrate (10X) with 990 mL of de-ionized or distilled water to prepare 1,000 mL of Wash Solution (1X).
Assay Procedure:	It is recommended that all Calibrators and Samples be added in duplicate to the Microtiter Plate. 1. Secure the desired number of coated wells in the holder, then add 100 µL of Calibrators or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. 2. Add 50 µL of Conjugate to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37°C. 3. Wash the microtiter plate using one of the specified methods indicated below: 4. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with wash solution, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of five washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. 5. Automated Washing: Aspirate all wells, then wash plate five times using wash solution. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes. 6. Add 50 µL substrate A and 50 µL substrate B to each well. Cover and incubate for 10 minutes at 20- 25°C. (Avoid exposure to light.) 7. Add 50 µL stop solution to each well. Mix well.

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	8. Read the optical density (OD) at 450 nm using a microtiter plate reader immediately.
Calculation of Results:	1. This calibration curve is used to determine the amount in an unknown sample. The calibration curve is generated by plotting the average OD (450 nm) obtained for each of the six calibrator concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis. 2. First, calculate the mean OD value for each calibrator and sample. All OD values are subtracted by the mean value of the zero calibrator before result interpretation. Construct the calibration curve using graph paper or statistical software. 3. To determine the amount in each sample, first locate the OD value on the Y-axis and extend a horizontal line to the calibration curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration. 4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in the result. Each user should obtain their own calibration curve. 5. The sensitivity by this assay is 0.1 ng/mL.
Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	All reagents provided are stored at 4°C. Refer to the expiration date on the label.
Expiry Date:	The expiry date is stated on the label.