Datasheet for ABIN612686

EGF ELISA Kit

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Overview

Quantity:	96 tests
Target:	EGF
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The AssayMax Human EGF ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human EGF in urine, saliva, milk, and cell culture samples
Brand:	AssayMax
Sample Type:	Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human EGF Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human EGF. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human EGF Standard: Human EGF in a buffered protein base (2 ng, lyophilized). Biotinylated EGF Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against EGF (140µl). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop

Product Details

	Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water.

Target Details

Target:	EGF
Abstract:	EGF Products
Background:	Human epidermal growth factor (EGF) is a mitogenic growth factor that plays important roles in cell growth, proliferation and differentiation. EGF is synthesized as a large precursor (1207 amino acids, 134 kDa) that is cleaved into a small mature protein (53 amino acids, 6 kDa). The precursor has 66% identity with the corresponding mouse protein (1-3). Its gene mutation causes autosomal recessive renal hypomagnesemia. EGF binds to the cell surface receptor EGFR, leading to the receptor tyrosine kinase phosphorylation and subsequent signal transduction pathways activation. The EGFR inhibition by small molecule tyrosine kinase inhibitors and monoclonal antibodies is the target of non-small cell lung cancer, colorectal cancer, pancreatic cancer, and breast cancer therapies (5-7).
Pathways:	NF-kappaB Signaling, RTK Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Regulation of Carbohydrate Metabolic Process, Hepatitis C, Protein targeting to Nucleus, Interaction of EGFR with phospholipase C-gamma, Thromboxane A2 Receptor Signaling, EGFR Downregulation

Application Details

Sample Volume:	50 µL
Assay Time:	< 5 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human EGF in less than 5 hours. A polyclonal antibody specific for human EGF has been pre-coated onto a 96-well microplate with removable strips. EGF in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for EGF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Application Details

Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIx Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 2 ng of EGF Standard with 2 ml of MIx Diluent to generate a solution of 1 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (1 ng/ml) 1:2 with MIx Diluent to produce 0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.0156 ng/ml solutions. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [EGF] (ng/ml) P1 Standard (1 ng/ml) 1.0000 P2 1 part P1 + 1 part MIx Diluent 0.5000 P3 1 part P2 + 1 part MIx Diluent 0.2500 P4 1 part P3 + 1 part MIx Diluent 0.1250 P5 1 part P4 + 1 part MIx Diluent 0.0625 P6 1 part P5 + 1 part MIx Diluent 0.0313 P7 1 part P6 + 1 part MIx Diluent 0.0156 P8 MIx Diluent 0.0000 Biotin EGF Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes. Dilute Urine 1:60 with MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Milk: Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Dilute Milk 1:20 with MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 2
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of EGF standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a

Application Details

	machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated EGF Antibody to each well and incubate for two hours. Wash the microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. 3 Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Restrictions:	For Research Use only

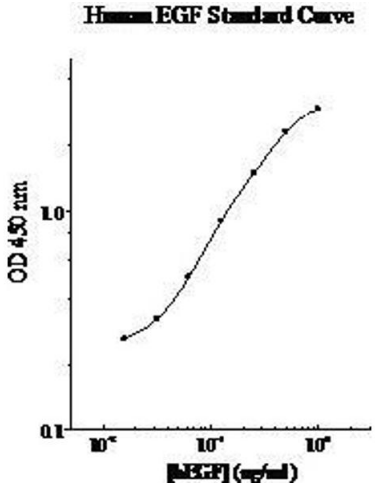
Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated- antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents. The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may

Handling

be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Images



ELISA

Image 1.