Datasheet for ABIN991630

NF-kB p65 ELISA Kit

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Overview

Quantity:	96 tests
Target:	NF-kB p65 (NFkBP65)
Reactivity:	Goat
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	This ELISA kit is a solid phase ELISA designed for quantitative determination.
Sample Type:	Cell Culture Supernatant, Plasma, Serum, Tissue Homogenate
Detection Method:	Colorimetric
Components:	• Microtiter plate (96 wells stripwell) - 1 • Enzyme conjugate - 1 vial • Standard A - 1 vial • Standard B - 1 vial • Standard C - 1 vial • Standard D - 1 vial • Standard E - 1 vial • Standard F - 1 vial • Substrate A - 1 vial • Substrate B - 1 vial • Stop solution - 1 vial • Wash solution - 1 vial • Balance solution - 1 vial • Instruction manual - 1

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Product Details

Material not included:	• Precision pipettors and disposable tips to deliver 10-1000µL. A multi-channel pipette is desirable for large assays. • 100mL and 1L graduated cylinders. • Distilled or deionized water • Tubes to prepare sample dilutions. • Absorbent paper. • Microplate reader capable of measuring absorbance at 450nm. • Centrifuge capable of 3000 × g. • Microplate washer or washing bottle. • Incubator (37°C). • Data analysis and graphing software.

Target Details

Target:	NF-kB p65 (NFkBP65)
Alternative Name:	Nuclear Factor Kb p65 (NFkBP65 Products)
Pathways:	NF-kappaB Signaling, RTK Signaling, TCR Signaling, TLR Signaling, Fc-epsilon Receptor Signaling Pathway, Neurotrophin Signaling Pathway, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Hepatitis C, Toll-Like Receptors Cascades, S100 Proteins

Application Details

Application Notes:	• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of samples used in the whole test. Please reserve sufficient amounts of samples in advance. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. • If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. • Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products. • Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

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Application Details

Comment:

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.
- The coefficient of determination of the standard curve should be higher or equal 0.95 and the highest O.D. should be more than 1.0.
- Cover or cap all kit components and store at 2-8°C when not in use.
- Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Do not mix or interchange different reagent lots from various kit lots.
- Do not use reagents after the kit expiration date.
- Read absorbance immediately after adding the stop solution.
- Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.

Information on standard material:

Different kits have different standards. For kits detecting proteisn or peptidse, the standards are recombinant proteins or synthetic peptides. For kits detecting small chemical compounds, the standards are synthetic chemical compounds. There are no standards extracted from natural resources. All of our reombinant proteins are expressed in E.coli. The standard are dissolved in PBS with 0.1 % proclin 300 and some other preservatives.

Information on reagents:

The STOP solution is 1M sulphuric acid. The wash buffer is 0.05 % Tween 20 in PBS, pH 7.4. The ELISA kit dose not contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME). Part of the reagents contain BSA.

Information on antibodies:

The provided antibodies and their host vary in different kits.

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Application Details

Sample Volume:	100 µL
Assay Time:	1.5 h
Plate:	Strips (12 x 8)
Protocol:	This ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal antibody for the target antigen and a target antigen HRP conjugate. The assay sample and buffer are incubated together with target antigen HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is inversely proportional to the target antigen concentration since the target antigen from samples and target antigen HRP conjugate compete for the antibody binding site. Since the number of sites is limited, as more sites are occupied by the target antigen from the sample, fewer sites are left to bind the conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The target antigen concentration in each sample is interpolated from this standard curve.
Reagent Preparation:	• **Samples** - Please predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution. PBS (pH 7.0-7.2) or 0.9% physiological saline can be used as dilution buffer. • **Wash solution** - Dilute 10mL of wash solution concentrate (100×) with 990mL of deionized or distilled water to prepare 1000mL of wash solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C. Note: • Bring all kit components and samples to room temperature (20-25°C) before use. • Do not dilute other ready-to-use components.
Sample Collection:	• **Serum**: Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000 × g (or 3000rpm) for 15 minutes. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. • **Plasma**: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 100 × g (or 3000rpm) at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. • **Tissue homogenates**: The preparation of tissue homogenates will vary depending upon tissue type. For this assay, thoroughly rinse tissues in ice-cold PBS (0.02mol/L, pH 7.0-7.2) to remove excess blood and weigh before homogenization. Mince the tissues into small pieces

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and homogenize them in a certain amount of PBS with a glass homogenizer on ice. Subject the resulting suspension to ultrasonication or to two freeze-thaw cycles to further break down cell membranes. After that, centrifuge for 15 minutes at 1500 × g (or 5000rpm). Remove the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

- **Cell lysates**: Cells should be lysed according to the following directions.
  - 1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
  - 2. Wash three times in PBS.
  - 3. Resuspend cells in PBS and subject to ultrasonication 3 times. Alternatively, freeze cells at -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.
  - 4. Centrifuge at 1000 × g (or 3000rpm) for 15 minutes at 2-8°C to remove cellular debris.
  - 5. Assay immediately or store samples at -20°C or -80°C.
- **Cell culture supernatants and other body fluids**: Centrifuge cell culture media at 1000 × g (or 3000rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

Note:

- Samples should be aliquoted and must be stored at -20°C (lower or equal 3 months) or -80°C (lower or equal 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2-8°C. Avoid repeated freeze-thaw cycles.
- Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Samples containing a visible precipitate must be clarified prior to use in the assay. Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
- Do not use heat-treated specimens.

Calculation of Results:

- The standard curve is used to determine the amount of samples.
- First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control well. DO NOT subtract the O.D. of standard zero.
- Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve using graph paper or statistical software to generate a four parameter logistic (4-PL) curve fit or logit log linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
- Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

Note:

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Application Details

- Any variation in operator, pipetting and washing technique, incubation time/temperature and kit age can cause variation in result. Each user should obtain their own standard curve.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- If specimen generate values higher than the highest standard, dilute the specimens and repeat the assay.

Restrictions: For Research Use only

Handling

Precaution of Use:

- This kit contains a small amount of 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.
- The stop solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.
- Care should be taken when handling the standard because of the known and unknown effects of it.
- Care should also be taken to avoid contact of skin or eyes with other kit reagents or specimens. In the case of contact, wash immediately with water.
- Do not pipette by mouth.
- Avoid generation of aerosols.
- Waste must be disposed of in accordance with federal, state and local environmental control regulations.
- All blood components and biological materials should be handled as potentially hazardous. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour a 121.5°C.

Handling Advice:

- The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
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degradation and denaturalization may occur in those samples and finally lead to wrong results.

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Storage:	4 °C
Expiry Date:	six months

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