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Datasheet for ABIN6968996

NF-κB p65 ELISA Kit

1 Image

Overview

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| Quantity: | 96 tests |
| Target: | NF-κB p65 (NFκBP65) |
| Reactivity: | Rat |
| Method Type: | Sandwich ELISA |
| Detection Range: | 78.125 pg/mL - 5000 pg/mL |
| Minimum Detection Limit: | 78.125 pg/mL |
| Application: | ELISA |

Product Details

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| Purpose: | For quantitative detection of NF-κB p65 in serum, plasma, tissue homogenates. |
| Sample Type: | Plasma, Serum, Tissue Homogenate |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This assay has high sensitivity and excellent specificity for detection of NF-κB p65. No significant cross-reactivity or interference between NF-κB p65 and analogues was observed. Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between NF-κB p65 and all the analogues, therefore, cross reaction may still exist. |
| Sensitivity: | 46.875 pg/mL |
| Components: | <ul style="list-style-type: none">• Pre-coated, ready to use 96-well strip plate |

Product Details

- Plate sealer for 96 wells
- Standard
- Sample/Standard Dilution Buffer
- Biotin-labeled Antibody (Concentrated)
- Antibody Dilution Buffer
- HRP-Streptavidin Conjugate (SABC)
- SABC Dilution Buffer
- TMB Substrate
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

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| Material not included: | <ol style="list-style-type: none">1. Microplate reader (wavelength:450nm)2. 37 °C incubator3. Automated plate washer4. Precision single and multi-channel pipette and disposable tips5. Clean tubes and Eppendorf tubes6. Deionized or distilled water |
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Target Details

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| Target: | NF-κB p65 (NFκBP65) |
| Alternative Name: | Nuclear Factor-kappaB P65 (NFκBP65 Products) |
| Background: | NF-κB p65, NFKB3, p65RelA, NFKB3v-rel avian reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65, Nuclear factor NF-kappa-B p65 subunit, Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 transcription factor p65, p65, v-rel reticuloendotheliosis viral oncogene homolog A, avian, v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 |
| UniProt: | Q7TQN4 |
| 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

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| Sample Volume: | 100 µL |
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Application Details

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| Plate: | Pre-coated |
| Protocol: | <ol style="list-style-type: none">1. Wash plate 2 times before adding Standard, Sample (diluted at least 1/2 with Sample Dilution Buffer) and Control (blank) wells!2. Add 100 μL standard or sample to each well and incubate for 90 minutes at 37 °C.3. Aspirate and wash plates 2 times.4. Add 100 μL Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37 °C.5. Aspirate and wash plates 3 times.6. Add 100 μL SABC Working Solution into each well and incubate for 30 minutes at 37 °C.7. Aspirate and wash plates 5 times.8. Add 90 μL TMB Substrate Solution. Incubate 10-20 minutes at 37 °C.9. Add 50 μL Stop Solution. Read at 450nm immediately and calculation. |
| Reagent Preparation: | <ul style="list-style-type: none">• Bring all reagents and samples to room temperature for 20 minutes before use.• Wash Buffer: If crystals have formed in the concentrate, you can warm it with 40 °C water bath Concentrated Wash Buffer into 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 2-8 °C.• Standards:<ol style="list-style-type: none">1. Add 1 mL Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly. Note: If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.2. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 mL of the Sample Dilution Buffer into each tube. Add 0.3 mL of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 mL from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 mL from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control. Note: It is best to use Standard Solutions within 2 hours.• Preparation of Biotin-labeled Antibody Working Solution:<p>Prepare it within 1 hour before experiment.</p><ol style="list-style-type: none">1. Calculate required total volume of the working solution: 0.1ml/well x quantity of wells. (Allow 0.1-0.2 mL more than the total volume.)2. Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 μL Biotin-labeled antibody into 99 μL Antibody Dilution Buffer.)• Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:<p>Prepare it within 30 minutes before experiment.</p><ol style="list-style-type: none">1. Calculate required total volume of the working solution: 0.1ml/well x quantity of wells. (Allow 0.1-0.2 mL more than the total volume.)2. Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 μL of SABC into 99 μL of SABC Dilution Buffer.) |
| Sample Preparation: | <ul style="list-style-type: none">• It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should |

therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution. The matrix components in the sample will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

Assay Procedure:

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 µL wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350 µL wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles, be sure the fluid can be sipped up completely)

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37 °C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples (diluted at least 1/2 with Sample Dilution Buffer), control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. Prepare Standards: Aliquot 100 µL of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube,

- 6thtube and Sample Dilution Buffer (blank) into the standard wells.
3. Add Samples: Add 100 μ L of properly diluted sample into test sample wells.
 4. Incubate: Seal the plate with a cover and incubate at 37 °C for 90 minutes.
 5. Wash: Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
 6. Biotin-labeled Antibody: Add 100 μ L Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37 °C for 60 minutes.
 7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.
 8. HRP-Streptavidin Conjugate (SABC): Add 100 μ L of SABC Working Solution into each well, cover the plate and incubate at 37 °C for 30 minutes.
 9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
 10. TMB Substrate: Add 90 μ L TMB Substrate into each well, cover the plate and incubate at 37 °C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
 11. Stop: Add 50 μ L Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
 12. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

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| Assay Precision: | Intra-Assay: CV<8% Inter-Assay: CV<10% |
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| Restrictions: | For Research Use only |
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Handling

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| Storage: | 4 °C, -20 °C |
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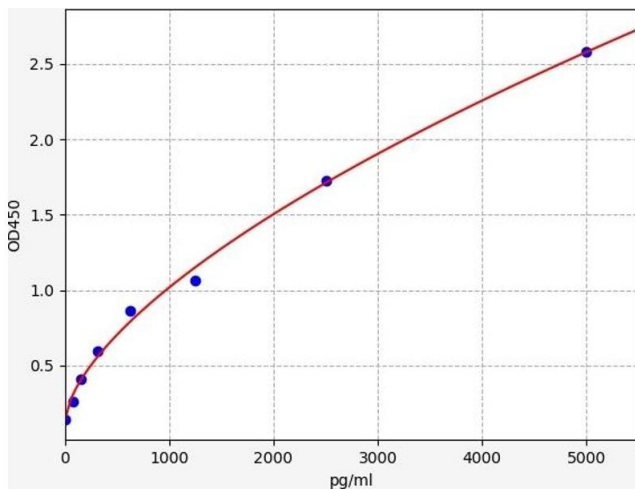
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| Storage Comment: | 1. For unopened kit: All the reagents should be kept according to the labels on vials. The Reference Standard and the 96-well stripe plate should be stored at -20 °C upon receipt while the other reagents should be stored at 4 °C. |
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Handling

2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

Expiry Date: 6 months

Images



ELISA

Image 1. Typical standard curve