Version 15-04/24

User's Manual

Bovine Nuclear Factor Kappa B ELISA Kit

REF **DEIA-PY2312**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This sandwich kit is for the accurate quantitative detection of Bovine Nuclear Factor Kappa B (also known as NFkB) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Principles of Testing

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Bovine NFkB antibody. NFkB present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Bovine NFkB Antibody is added and binds to NFkB in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated NFkB antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Bovine NFkB. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents And Materials Provided

1. Standard Solution (24ng/ml): 0.5ml x1
2. Pre-coated ELISA Plate: 12 * 8 well strips x1
3. Standard Diluent: 3ml x1
4. Streptavidin-HRP: 6ml x1
5. Stop Solution: 6ml x1
6. Substrate Solution A: 6ml x1
7. Substrate Solution B: 6ml x1
8. Wash Buffer Concentrate: (30x) 20ml x1
9. Biotinylated Bovine NFkB Antibody: 1ml x1
10. User Instruction: 1
11. Plate Sealer: 2 pics
12. Zipper bag: 1 pic

Materials Required But Not Supplied

1. 37°C±0.5°C incubator
2. Absorbent paper
3. Precision pipettes and disposable pipette tips
4. Clean tubes
5. Deionized or distilled water
6. Microplate reader with 450 ± 10nm wavelength filter

Storage

Store the reagents at 2-8°C. For over 6-month storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month

Specimen Collection And Preparation

Serum

Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2 - 8°C within 30 minutes of collection.

Urine

Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

Cell Culture Supernatant

Collect by sterile tubes when examining secrete components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Tissue

Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Note:

(1) Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for their particular experiments.

(2) Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.

(3) Samples should be brought to room temperature before starting the assay.

(4) Centrifuge to collect sample before use.

(5) Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

(6) Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

(7) Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Reagent Preparation

All reagents should be brought to room temperature before use.

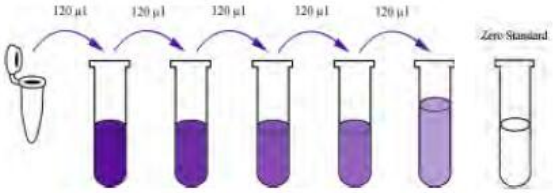
Wash Buffer

Dilute 20ml of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Standard

Reconstitute the 120µl of the standard (24ng/ml) with 120µl of standard diluent to generate a 12ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (12ng/ml) 1:2 with standard diluent to produce 6ng/ml, 3ng/ml, 1.5ng/ml and 0.75ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

12ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
6ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
3ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
1.5ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
0.75ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
24ng/ml	12ng/ml	6ng/ml	3ng/ml	1.5ng/ml	0.75ng/ml

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-NFkB antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

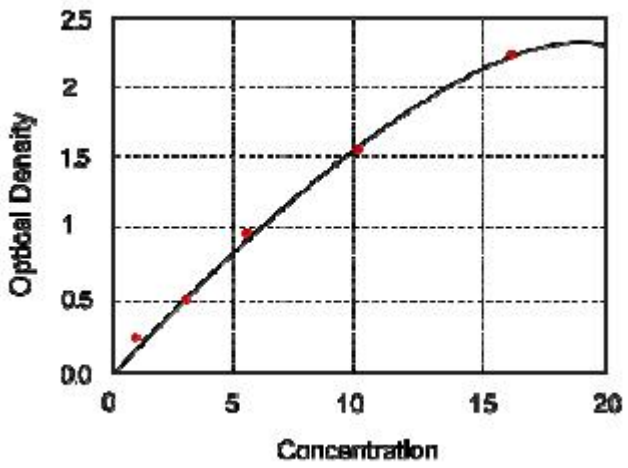
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Calculation

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Precision

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in separate assays to assess inter-assay precision.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

0.05ng/ml - 20ng/ml

Sensitivity

0.024ng/ml

Precautions

1. Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
2. This instruction must be strictly followed in the experiment.
3. Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
4. Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
5. Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
6. Avoid using the reagents from different batches together.
7. Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
8. Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
9. The kit should not be used beyond the expiration date.