



Sino Biological
Biological Solution Specialist

Human I κ B alpha / NF κ BIA ELISA Pair Set

Catalog Number : SEK12045

To achieve the best assay results, this manual must be read carefully before using this product and the assay is run as summarized in the General ELISA protocol.

BACKGROUND

Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I κ B α , NFKBIA, or IKBA), is a member of the NF-kappa-B inhibitor family that function to inhibit the NF-kB transcription factor. NFKBIA inhibits NF-kB by masking the nuclear localization signals (NLS) of NF-kB proteins and keeping them sequestered in an inactive state in the cytoplasm. In addition, NFKBIA blocks the ability of NF-kB transcription factors to bind to DNA, which is required for NF-kB's proper functioning. Signal-induced degradation of I kappa B α exposes the nuclear localization signal of NF-kappa B, thus allowing it to translocate into the nucleus and activate transcription from responsive genes. An autoregulatory loop is established when NF-kappa B induces expression of the I kappa B α gene and newly synthesized I kappa B α accumulates in the nucleus where it negatively regulates NF-kappa B-dependent transcription. As part of this post-induction repression, the nuclear export signal on I kappa B α mediates transport of NF-kappa B-I kappa B α complexes from the nucleus to the cytoplasm. Deletion of NFKBIA has an effect that is similar to the effect of EGFR amplification in the pathogenesis of glioblastoma and is associated with comparatively short survival. Polymorphisms in NFKBIA may be important in pre-disposition to and outcome after treatment, of multiple myeloma (MM). The NFKBIA gene product, IkappaB α , binds to NF-kappaB preventing its activation and is important in mediating resistance to apoptosis in B-cell lymphoproliferative diseases.

PRINCIPLE OF THE TEST

The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for IκB alpha / NFκBIA coated on a 96-well plate. Standards and samples are added to the wells, and any IκB alpha / NFκBIA present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-IκB alpha / NFκBIA monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of IκB alpha / NFκBIA present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- ◆The Human IκB alpha / NFκBIA ELISA Pair Set is for the quantitative determination of Human IκB alpha / NFκBIA.
- ◆This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This Pair Set has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 1.0 mg/mL of rabbit anti-NFKBIA monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in CBS before coating. (Catalog: # 12045-R056)

Detection Antibody – 0.5 mg/mL mouse anti-NFKBIA monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % glycerol, pH 7.4). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use. (Catalog: # 12045-MM02)

Standard – Each vial contains 22 ng of recombinant NFKBIA. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 0.8 ng/mL is recommended.

SOLUTIONS REQUIRED

CBS - 0.05M Na₂CO₃ , 0.05M NaHCO₃ , pH 9.6, 0.2 µm filtered

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na₂HPO₄ and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H₂O₂ , mix it well

Stop Solution - 2 N H₂SO₄

PRECAUTION

The Stop Solution suggested for use with this Pair Set is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Protect it from prolonged exposure to light. Aliquot and store at -20°C to -80°C and for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

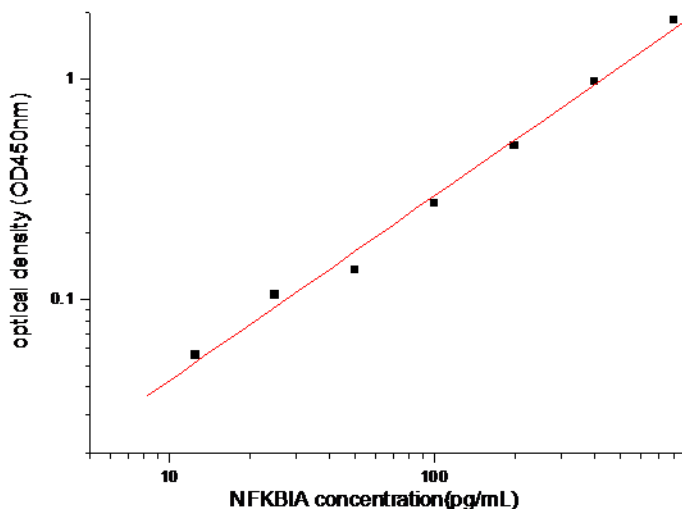
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (**if substrate solution is not as requested, the incubation time should be optimized**). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

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



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
12.5	0.056
25	0.105
50	0.136
100	0.272
200	0.499
400	0.978
800	1.851

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human I κ B alpha / NFKBIA was determined to be approximately 12.5 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Notes