



TECHNICAL MANUAL

Mouse Inhibitory Subunit Of NF Kappa B Alpha (IkBa) ELISA Kit

- **SKU CODE:** MODL00670
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Mouse Inhibitory Subunit Of NF Kappa B Alpha (IkBa) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

Table of Contents

1. Key Features	3
2. Storage & Expiry	3
3. Product Description	4
4. Kit Contents	5
5. Precautions	5
6. Assay Summary	6
7. Sample Preparation	7
8. Standard and Reagent Preparation	11
9. Assay Procedure	14
10. Data Analysis	15
11. Typical Data	16
12. ELISA Troubleshooting	18

1. Key Features

Detection Method:

Sandwich

Sample Type:

Tissue homogenates, cell lysates and other biological fluids

Reactivity:

Mouse

Range:

0.312-20 ng/mL

Sensitivity:

< 0.113 ng/mL

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Date of expiration is on the ELISA Box label. Storage conditions are provided below:

- **Storage of Unopened Kits:** All reagents must be kept store according to labels on the vials. The **TMB Substrate, Wash Buffer (30x concentrate) and the Stop Solution** should be stored at **4 °C** upon receipt. The rest of reagents should be stored at **-20 °C** immediately upon receipt.
- **Storage of Opened Kits:** After opening, all remaining reagents must continue to be stored under the same conditions specified above. Unused wells must be returned to the foil pouch containing the desiccant pack. The pouch should then be resealed securely along the entire edge of the zip-seal to maintain integrity. **Note:** *It is recommended to use the reagents within 1 month of opening.*

3. Product Description

The Assay Genie Mouse Inhibitory Subunit Of NF Kappa B Alpha (I κ B α) ELISA Kit is a highly sensitive assay for the quantitative measurement of I κ B α in the following samples: tissue homogenates, cell lysates and other biological fluids.

This kit utilizes a sandwich enzyme-linked immunosorbent assay (ELISA) format. An antibody specific to the target protein is pre-coated onto the wells of a 96-well microplate. Samples, standards, and blanks are added to antibody-precoated wells and incubated for antigen binding. After washing, a biotin-conjugated detection antibody (Detection Reagent A) is added, followed by a second incubation and wash. An HRP-avidin conjugate (Detection Reagent B) is then added, incubated, and washed away. TMB substrate is added and reacts with HRP enzyme. The reaction produces a blue product that turns yellow upon addition of the acidic Stop Solution.

The optical density (OD) is measured at 450 nm using a microplate reader. The OD₄₅₀ value is directly proportional to the concentration of the target analyte in the sample, which can be determined by referencing a standard curve.

4. Kit Contents

No	Component Name	Specifications
1	Pre-coated, ready to use 96-well strip plate	1
2	Standard	2
3	Detection Reagent A	1 × 120 µL
4	Detection Reagent B	1 × 120 µL
5	TMB Substrate	1 × 9 mL
6	Stop Solution	1 × 6 mL
7	Wash Buffer (30× concentrate)	1 × 20 mL
8	Diluent Buffer	1 × 45 mL
9	Plate sealer for 96 wells	2
10	Instruction manual	1

Additional materials required:

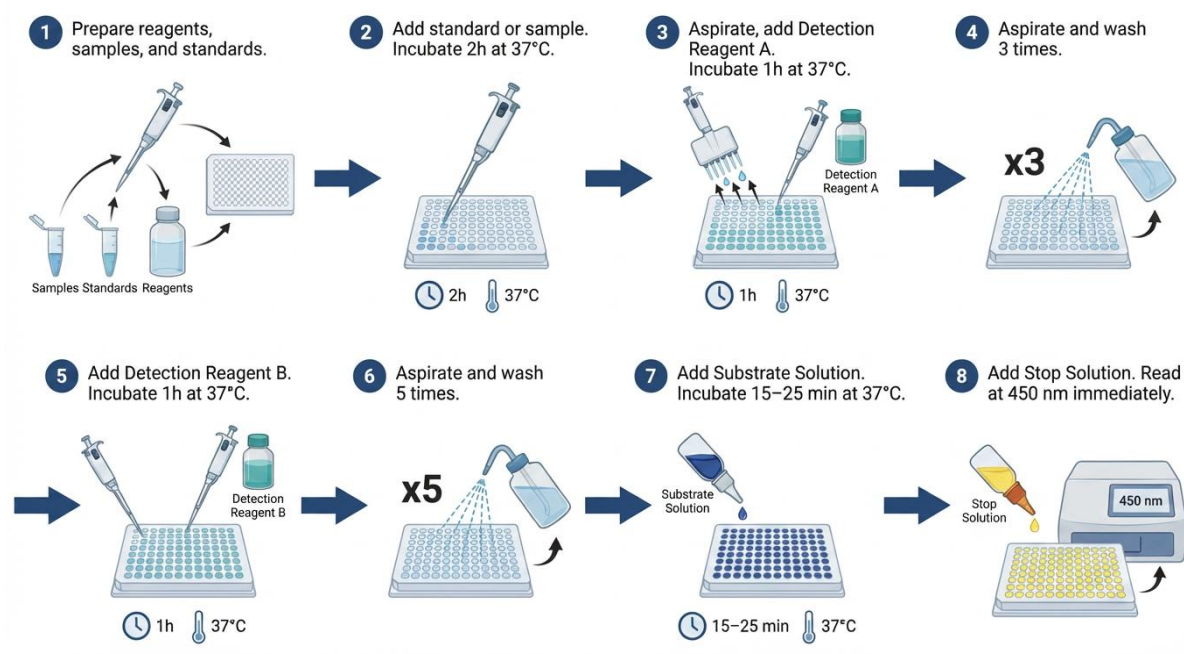
1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. This kit is for research purposes only and not for diagnostics or therapeutic uses.
2. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Ensure unopened and unused plates are kept dry to avoid contamination.
5. Before using the kit, centrifuge tubes to spin down standard and/or antibody.
6. Prepare all reagents, samples and standards as directed in this manual.

7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during the assay.
9. Maintain consistent incubation times and temperatures as variations can affect results.
10. Do not reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.

6. Assay Summary



7. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

Note: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions:**
 - **Short-term:** 2-8 °C for up to 5 days.
 - **Medium-term:** -20 °C for up to 1 month.
 - **Long-term:** -80 °C for up to 2 months.
- **Thawing Protocol:** Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles:** Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

A. Tissue Homogenates

Tissue samples should be homogenized prior to use. Avoid buffers containing NP-40, Triton X-100, or DTT, as these strongly inhibit the assay. We recommend using PBS (0.01 mol/L, pH 7.0-7.2).

The recommended protocol is as follows:

- **Sample Collection and Washing**

- Place the target tissue on ice.
- Rinse the tissue with pre-cooled PBS buffer (0.01 M, pH 7.0-7.2) to remove residual blood.
- Weigh the tissue for further processing.
- **Homogenization**
 - Mince the tissue into small pieces and homogenize it in PBS on ice using a glass homogenizer (micro tissue grinders also work).
 - The lysate volume should correspond to the tissue weight; typically, 9 mL PBS is used per 1 g of tissue. It is recommended to add protease inhibitors to the PBS (e.g., 1 mM PMSF). **Note:** *Avoid chemical lysis buffers, as certain chemicals may cause unexpected ELISA results.*
- **Cell Disruption**
 - Further disrupt the tissue using ultrasonic homogenization or freeze–thaw cycles.
 - Ultrasonic homogenization: Keep samples on an ice bath during sonication to avoid overheating.
 - Freeze–thaw cycles: Repeat twice for effective lysis.
- **Centrifugation and Storage**
 - Centrifuge the homogenate at 5000 × g for 5 minutes.
 - Collect the supernatant for immediate analysis, or aliquot and store at –20°C or –80°C for future assays.
- **Protein Concentration Measurement**
 - Determine total protein concentration.
 - For ELISA assays, the total protein concentration should generally be 1–3 mg/mL.
 - Tissues with high endogenous peroxidase levels (e.g., liver, kidney, pancreas) may react with TMB substrate, causing false positives. If this occurs, treat samples with 1% H₂O₂ for 15 minutes before repeating the assay.

Note: Liver, kidney, and pancreas samples often contain high levels of endogenous peroxidase, which may react with the chromogenic substrate at elevated sample concentrations, potentially resulting in false positive signals.

If analysis of these tissues is required, a gradient dilution assay is recommended. A proportional decrease in signal with increasing dilution typically indicates minimal interference and supports the accuracy of the results.

To further minimise potential interference, samples can be pre-treated with 1% hydrogen peroxide (H_2O_2) for 15 minutes prior to testing. To prepare the treatment solution, add 1 μ l of pure H_2O_2 to 100 μ l of sample (1% v/v).

B. Other Biological Fluids

Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately, or aliquot and store at –20°C or –80°C for future assays. Avoid repeated freeze/thaw cycles.

C. Cell Lysates

- **Suspension Cell Lysate:** Collect the cells by centrifugation and wash them 3 times in cold PBS. Resuspend the cells in PBS (1×) and subject them to ultrasonication 4 times (or freeze the cells at ≤ –20°C, thaw with gentle mixing and repeat the freeze/thaw cycle 3 times).
- **Adherent Cell Lysate:** Detach the cells with trypsin and collect them by centrifugation. Wash the cells 3 times in cold PBS. Resuspend the cells in PBS (1×) and subject them to ultrasonication 4 times (or freeze the cells at ≤ –20°C, thaw with gentle mixing and repeat the freeze/thaw cycle 3 times).

Follow next steps for protein extraction and supernatant collection:

- **Protein Release and DNA Disruption**
 - During lysis, pipette gently or intermittently shake the tube to enhance protein extraction.

- Mucilaginous material formed during lysis is DNA, which can be broken down by ultrasonic disruption (3–5 mm probe, 150–300 W, 3–5 seconds per cycle, with 30-second intervals for 1–2 minutes total).
- **Supernatant collection**
 - After lysis or ultrasonic treatment, centrifuge the lysate at $1500 \times g$ for 10 minutes at 2–8°C to remove cellular debris. Collect the supernatant for immediate use or aliquot and store at –80°C for future assays.

Notes: Refer to the "Tissue Sample Notes" for additional buffer and inhibitor recommendations.

D. Other Sample Types

For more information about how to process other sample types, (e.g., breast milk & more), please contact our Tech Support Team at techsupport@assaygenie.com.

8. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 μ l wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350 μ l wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute.

Note: Set the height of the needles; be sure the fluid can be taken up completely.

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

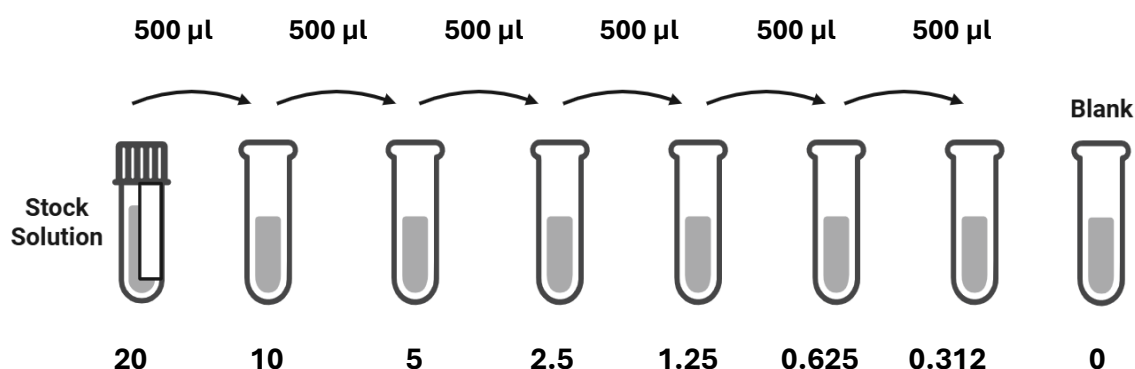
Note: Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution. Samples can also be diluted with 0.01 mol/L PBS (pH 7.0-7.2) in case of insufficient Diluent Buffer.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

A. Standard Dilution:

1. Reconstitute the Standard with 1.0 mL of Diluent Buffer, let it stand for 10 min at room temperature and invert it gently several times. This reconstitution produces a working solution of 20 ng/mL. Prepare 7 tubes containing 500 μ L Diluent Buffer and use the diluted standard to produce a double dilution series according to the image shown below. Prepare a dilution series with 7 points, for example: 20, 10, 5 2.5, 1.25, 0.625, 0.312 and 0 ng/mL. **Note:** The final tube serves as the blank and should not receive any solution transferred from the preceding tube.



B. Preparation Detection Reagent A and Detection Reagent B:

1. Briefly spin or centrifuge the stock Detection A and Detection B solutions before use.
2. Dilute to the working concentrations with Diluent Buffer respectively (1:100)

C. Wash Solution:

1. Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).

D. TMB Substrate:

1. Aspirate the required volume of the solution using sterile pipette tips. Do not return any unused or residual solution to the original vial.

Notes:

1. *Do not perform a serial dilution directly in the wells.*
2. *Prepare standard within 15 minutes of performing the assay. Do not dissolve the reagents at 37°C directly.*
3. *Detection Reagent A and B are sticky solutions. Slowly pipette them to reduce volume errors. It is recommended to pipette more than 10 µL at a time to ensure accuracy.*
4. *Carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, avoid foaming and mix gently until the crystals are completely dissolved.*
5. *The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.*
6. *If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.*
7. *Any contaminated water or container used during reagent preparation will influence the detection result.*

9. Assay Procedure

1. **Plate Setup:** Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. **Standard, Samples & Control Loading:** Aliquot 100 µl of standard working solution or samples and controls into the designated wells. **Note:** *Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.*
3. **First Incubation:** Seal the plate with a cover and incubate at 37 °C for 2 hours.
4. **Aspirate:** Aspirate the liquid from each well, DO NOT wash.
5. **Detection Reagent A Addition:** Add 100 µl of Detection Reagent A working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.
6. **Second Incubation:** Seal the plate with a cover and incubate at 37°C for 1 hour.
7. **Washing:** Aspirate or decant the solution from the plate and add 350 µL of 1x Wash Solution to each well and incubate for 1-2 minutes at room temperature. Aspirate the solution from each well and clap the plate on absorbent filter paper to dry. Repeat this process three times. **Note:** *A microplate washer can be used in this step and other wash step.*
8. **Detection Reagent B Addition:** Add 100 µL of Detection Reagent B working solution to each well.
9. **Third incubation:** Cover with a plate seal and incubate for 1 hour at 37°C.
10. **Wash:** Aspirate or decant the solution from each well. Repeat the wash process five times as conducted in step 7.
11. **Substrate Reagent Addition and Colour Development:** Add 90 µl of Substrate Reagent into each well, cover the plate and incubate at 37°C in dark for 15-25 mins. (**Note:** *This incubation time is for reference only, the optimal time should be determined by the end-user. DO NOT exceed 30 minutes*).

12. **Stop Reaction:** Add 50 μ l of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately. **Note:** *Stop solution addition should be done in the same order as the substrate solution.*
13. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

10. Data Analysis

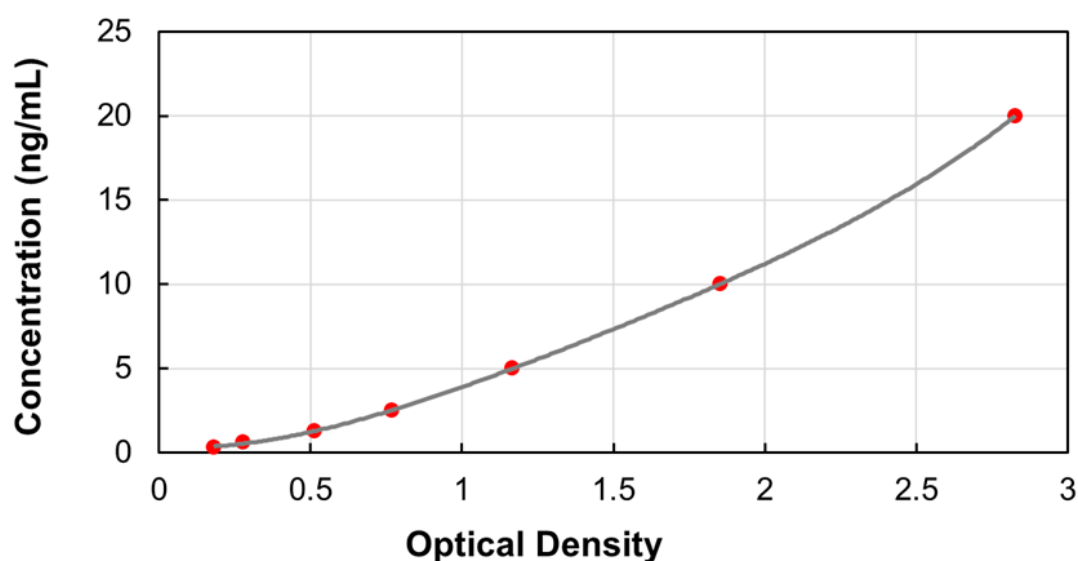
Average the duplicate readings for each standard, control, and sample, then subtract the mean optical density of the zero standard. Construct a standard curve by plotting IkBa concentration on the y-axis against absorbance on the x-axis, and fit the data using an appropriate best-fit curve.

For diluted samples, multiply the concentration obtained from the standard curve by the corresponding dilution factor to determine the final concentration. Data analysis and curve fitting may be performed using suitable plotting software (e.g., CurveExpert).

11. Typical Data

Standard Curve

Results of a typical standard run of an ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



Detection Range

0.312-20 ng/mL. The concentrations used for creating the standard curve were 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL and 0.312 ng/mL.

Sensitivity

The minimum detectable concentration of IkBa is typically less than 0.113 ng/mL. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of IκBa and their serial dilutions. No significant cross-reactivity or interference between IκBa and analogs was observed.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level IκBa were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level IκBa were tested on 3 different plates, 8 replicates in each plate.

$$CV (\%) = SD/mean \times 100$$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

12. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution.	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



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