**Catalog Number: N/A  
Lot Number: SAMPLE**

## INTENDED USE

This kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of LMNB2 in human tissue homogenates, cell lysates and other biological fluids.  
REAGENTS AND MATERIALS PROVIDED  
MATERIALS REQUIRED BUT NOT SUPPLIED  
Microplate reader with 450 ± 10 nm filter.  
Precision single or multi-channel pipettes and disposable tips.  
Eppendorf Tubes for diluting samples.  
Deionized or distilled water.  
Absorbent paper for blotting the microtiter plate.  
Container for Wash Solution.  
STORAGE OF THE KITS  
For unopened kits: All the reagents should be stored at -20°C upon receipt.  
For opened kits: Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.  
Note:  
For the expiration date of the kit, please refer to the label on the kit box. All components are stable until this date.  
It is highly recommended to use the remaining reagents within 1 month of opening.  
SAMPLE COLLECTION AND STORAGE  
Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues should be rinsed in ice-cold PBS (0.01 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Mince the tissues to small pieces and homogenize them in 5-10 mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders also work). The resulting suspension should be sonicated with an ultrasonic cell disrupter or subjected to 2 freeze/thaw cycles to further break the cell membranes. Then, centrifuge the homogenates for 5 minutes at 5000 × g. Remove the supernatant and assay immediately or aliquot and store at ≤-20°C.  
Cell Lysates - Cells must be lysed before assaying according to the following directions.  
Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly).  
Wash cells 3 times in cold PBS.  
Resuspend cells in PBS (1×) and subject the cells to ultrasonication 4 times (or Freeze cells at ≤-20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.).  
Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris.  
Other biological fluids - Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquots at -20°C or -80°C. Avoid repeated freeze/thaw cycles.  
Note:  
Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and/or contamination.  
Noticeable hemolysis will affect antibody-antigen reactions. Samples with any sign of hemolysis are not acceptable for this assay.  
When performing the assay, bring samples to room temperature.

## ASSAY PRINCIPLE

The microtiter plate provided in this kit has been pre-coated with an antibody specific to LMNB2. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific to LMNB2. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain LMNB2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 ± 10 nm. The concentration of LMNB2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.  
CALCULATION OF RESULTS  
Average the duplicate readings for each standard, control and sample, then subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with LMNB2 concentration on the y-axis and absorbance on the x-axis. Using plotting software, (for instance, curve expert 1.30), is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.  
TYPICAL DATA  
To make the calculation easier, we plot the O.D. value of the standard (x-axis) against the known concentration of the standard (y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique or temperature effects), plotting the log of the data to establish a standard curve for each test is recommended. The typical standard curve below is provided for reference only.  
DETECTION RANGE  
0.156-10 ng/mL. The concentrations used for creating the standard curve were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, 0.156 ng/mL.  
SENSITIVITY  
The minimum detectable dose of LMNB2 is typically less than 0.051 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 LMNB2.  
No significant cross-reactivity or interference between LMNB2 and analogues was observed.  
Note:  
Limited by current skills and knowledge, it is impossible to perform all possible cross-reactivity detection tests between LMNB2 and all analogues, therefore, cross reactivity may still exist.  
PRECISION  
Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level LMNB2 were tested 20 times on one plate, respectively.  
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level LMNB2 were tested on 3 different plates, 8 replicates in each plate.  
CV (%) = SD/mean × 100  
Intra-Assay: CV10%  
Inter-Assay: CV12%  
STABILITY  
The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage conditions.  
Note:  
To minimize unnecessary influences on the performance, operation procedures and lab conditions, especially room temperature, air humidity, and incubator temperatures should be strictly regulated. It is also strongly suggested that the whole assay is performed by the same experimenter from the beginning to the end.  
ASSAY PROCEDURE SUMMARY  
Prepare all reagents, samples and standards.  
Add 100 µL standard or sample to each well. Incubate 90 minutes at 37°C.  
Aspirate and add 100 µL Detection Solution A. Incubate 45 minutes at 37°C.  
Aspirate and wash 3 times.  
Add 100 µL Detection Solution B. Incubate 45 minutes at 37°C.  
Aspirate and wash 5 times.  
Add 90 µL Substrate Solution. Incubate 15-25 minutes at 37°C.  
Add 50 µL Stop Solution. Read at 450 nm immediately.  
IMPORTANT NOTE  
Limited by the current conditions and scientific technology, it is impossible to conduct comprehensive identification and analysis tests on the raw materials provided by suppliers. As a result, it is possible there are some qualitative and/or technical risks.  
The final experimental results will be closely related to the validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available to obtain accurate results.  
Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction manual included in your kit. The electronic ones on our website are for reference only.  
Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by the manufacturer.  
Protect all reagents from strong light during storage and incubation. All bottle caps of reagents should be closed tightly to prevent evaporation of liquids and contamination by microorganisms.  
There may be a foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.  
Incorrect procedures during reagent preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10 nm wavelength is acceptable for use in absorbance measurement. Please read the instructions carefully and adjust the instrument prior to the experiment.  
Even the same experimenter may get different results from two separate experiments. To get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before the general assay for each batch is recommended.  
Each kit has undergone several rigorous quality control tests. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance among kits from different batches could arise from the above factors as well.  
Kits from different manufacturers with the same item might produce different results since we have not compared our products with other manufacturers.  
The standard in this kit, as well as the antigens used in antibody preparation are typically recombinant proteins. Differently expressed sequences, expression systems, and/or purification methods can be used in the preparation of recombinant proteins. There is also the possibility of differences in the screening technique of antibodies and antibody pairs in our kits. As a result, we cannot guarantee that our kit will be able to detect recombinant proteins produced by other companies. We do NOT recommend using Innovative Research ELISA kits for the detection of other recombinant proteins.  
Validity period: 16 months.  
The instruction manual also works with the 48 T kit, but all reagents in the 48 T kit are reduced by half.  
PRECAUTION  
The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this reagent.

## TECHNICAL DETAILS

|  |  |
| --- | --- |
| Parameter | Characteristics |
| Detection Range | 62.5 pg/ml - 4,000 pg/ml |
| Sensitivity | 12 pg/ml |
| Specificity | Natural and recombinant Mouse Klk1 |
| Cross Reactivity | This kit is for the detection of Mouse Klk1. No significant cross-reactivity or interference between Klk1 and its analogs was observed. |

## OVERVIEW

|  |  |
| --- | --- |
| Sample Type | Detection Information |
| Serum Plasma |  |
| Cell Culture Supernatant |  |
| Other Biological Fluids |  |

## BACKGROUND

Kallikreins are a group of serine proteases with diverse physiological functions.   
 Kallikrein 1 (KLK1) is a tissue kallikrein that is primarily expressed in the kidney, pancreas, and salivary glands.  
 It plays important roles in blood pressure regulation, inflammation, and tissue remodeling through the kallikrein-kinin system.  
 KLK1 specifically cleaves kininogen to produce the vasoactive peptide bradykinin, which acts through bradykinin receptors to mediate various biological effects.  
 Studies have implicated KLK1 in cardiovascular homeostasis, renal function, and inflammation-related processes.

## KIT COMPONENTS

|  |  |
| --- | --- |
| Component | Quantity |
|  |  |

## MATERIALS REQUIRED BUT NOT PROVIDED

## STORAGE

## SAMPLE COLLECTION AND STORAGE

## REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.  
  
Standard - Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (avoid bubbles). The concentration of the standard in the stock solution is 10 ng/mL. Prepare 7 tubes containing 0.5 mL Standard Diluent and use the diluted standard to produce a double dilution series . Mix each tube thoroughly before the next transfer. Prepare a dilution series with 7 points, for example: 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.312 ng/mL, 0.156 ng/mL, and the last EP tube with Standard Diluent is the blank at 0 ng/mL.  
  
Detection Solution A and Detection Solution B - Detection Solutions A and B are already at the correct concentrations and do not need to be diluted further.  
  
Wash Solution - Dilute 20 mL of Wash Solution concentrate (30×) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).  
  
TMB substrate - Aspirate the needed dosage of the solution with sterilized tips. Do not dump the residual solution back into the vial.  
  
Note:  
  
Do not perform a serial dilution directly in the wells.  
  
Prepare standard within 15 minutes of performing the assay.  
  
Carefully reconstitute Standards according to the instruction, avoid foaming and mix gently until the crystals are completely dissolved.  
  
The reconstituted Standards can be used only once.  
  
If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.  
  
Any contaminated water or container used during reagent preparation will influence the detection result.

## ASSAY PROCEDURE

[' Determine wells for diluted standard, blank and sample. Prepare 7 wells for the standards, 1 well for blank. Add 100 μL each of dilutions of standard (read Reagent Preparation), blank, and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 90 minutes at 37°C. Remove the liquid from each well. Add 100 μL of Detection Solution A to each well. Incubate for 45 minutes at 37°C after covering it with the Plate sealer. Aspirate the solution and wash with 300 μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by tapping the plate onto absorbent paper. Wash thoroughly 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. Add 100 μL of Detection Solution B to each well. Incubate for 45\xa0minutes at 37°C after covering it with the Plate sealer. Repeat the aspiration/wash process for a total of 5 times as conducted in step 4. Add 90 μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-25 minutes at 37°C (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of the Substrate Solution. Add 50 μL of Stop Solution to each well. The liquid will turn yellow with the addition of the Stop solution. Mix the liquid by tapping the side of the plate. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing. Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Run the microplate reader and take measurements at 450 nm immediately. Note: Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remaining wells should be resealed and stored at -20°C. Samples or reagents addition: Please use the freshly prepared Standard. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. In addition, use separated reservoirs for each reagent.']

## DATA ANALYSIS

Average the duplicate readings for each standard, control and sample, then subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with LMNB2 concentration on the y-axis and absorbance on the x-axis. Using plotting software, (for instance, curve expert 1.30), is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. TYPICAL DATA

## DISCLAIMER

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.  
  
This kit is manufactured by Boster Biological Technology. Innovative Research is the exclusive distributor of this product. The product is warranted to perform as described in the accompanying protocol. If this product does not perform as described in our published materials, please contact Innovative Research for replacement.