# **Mouse KLK1/Kallikrein 1 ELISA Kit**

Catalog Number: IMSKLK1KT  
Lot Number: 20250424

## **INTENDED USE**

For the quantitation of Mouse Klk1 concentrations in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

## **TECHNICAL DETAILS**

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| --- | --- |
| **Capture/Detection Antibodies** | Rat monoclonal / Goat polyclonal |
| **Specificity** | Natural and recombinant Mouse Klk1 |
| **Standard Protein** | Recombinant Mouse Klk1 |
| **Cross-reactivity** | No detectable cross-reactivity with other relevant proteins |
| **Sensitivity** | <2 pg/ml |

## **OVERVIEW**

## **BACKGROUND**

Kallikrein-1, also known as tissue kallikrein, is a protein that in humans is encoded by the KLK1 gene. This serine protease generates Lys-bradykinin by specific proteolysis of kininogen-1. KLK1 is a member of the peptidase S1 family. Its gene is mapped to 19q13.3. In all, it has got 262-amino acids which contain a putative signal peptide, followed by a short activating peptide and the protease domain. The protein is mainly found in kidney, pancreas, and salivary gland, showing a unique pattern of tissue-specific expression relative to other members of the family. KLK1 is implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers.

## **ASSAY PRINCIPLE**

The Innovative Research Mouse Klk1 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Mouse Klk1 with a 96-well strip plate that is pre-coated with antibody specific for Klk1. The detection antibody is a biotinylated antibody specific for Klk1. The capture antibody is monoclonal antibody from rat and the detection antibody is polyclonal antibody from goat. The kit includes Mouse Klk1 protein as standards. To measure Mouse Klk1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Mouse Klk1 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Klk1 in the sample.

## **KIT COMPONENTS**

|  |  |  |  |
| --- | --- | --- | --- |
| **Description** | **Quantity** | **Volume** | **Storage** |
| Anti-Mouse Klk1 Pre-coated 96-well Strip Microplate | 1 | 12 strips of 8 wells | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Mouse Klk1 Standard | 2 | 10 ng/tube | Discard the Klk1 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours. |
| Mouse Klk1 Biotinylated Antibody (100x) | 1 | 100 µl | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 | 100 µl | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Sample Diluent | 1 | 30 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Antibody Diluent | 1 | 12 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Avidin-Biotin-Peroxidase Diluent | 1 | 12 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Color Developing Reagent (TMB) | 1 | 10 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Stop Solution | 1 | 10 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Wash Buffer (25x) | 1 | 20 ml | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Plate Sealers | 4 | Piece |  |

## **MATERIALS REQUIRED BUT NOT PROVIDED**

* Microplate reader capable of reading absorbance at 450 nm. Incubator.
* Automated plate washer (optional)
* Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for a large numbers of samples.
* Deionized or distilled water. 500 ml graduated cylinders. Test tubes for dilution.
* Mouse Klk1 ELISA Standard Curve Example
* The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

## **REAGENT PREPARATION**

Bring all reagents to room temperature before use. Wash Buffer: Dilute Wash Buffer (25X) with distilled water. For example, if preparing 500 ml of Wash Buffer, dilute 20 ml of Wash Buffer (25X) into 480 ml of distilled water. Standard: Reconstitute the standard with standard diluent according to the label instructions. This reconstitution produces a stock solution. Let the standard stand for a minimum of 15 minutes with gentle agitation prior to making dilutions. Detection Reagent A and B: Dilute to the working concentration using Assay Diluent A and B, respectively.

## **DILUTION OF STANDARD**

Dilute the standard stock solution in standard diluent buffer to concentrations of 62.5, 125, 250, 500, 1000, 2000, and 4000 pg/ml. A 7-point standard curve is recommended.

## **PREPARATIONS BEFORE ASSAY**

1. Prepare all reagents, samples, and standards according to the instructions.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Set all reagents to room temperature before beginning the assay.

## **SAMPLE PREPARATION AND STORAGE**

When first using a kit, appropriate validation steps should be taken to ensure the kit performs as expected.

## **SAMPLE COLLECTION NOTES**

Boster recommends that samples are used immediately upon preparation.

## **SAMPLE DILUTION GUIDELINE**

* To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
* The TMB Color Developing agent is colorless and transparent before using, contact us if it is not the case.
* The Standard solution should be clear and colorless or a very light yellow before use.

## **ASSAY PROTOCOL**

1. Aliquot
2. 1ml per well of the dilutions of the standard, blank, and samples into the pre-coated 96-well plate.
3. Seal the plate with a cover and incubate at 37°C for 90 min.
4. Remove the cover, discard plate contents, and blot the plate onto paper towels.
5. Add
6. 1ml of biotinylated anti-Mouse Klk1 antibody working solution into each well and incubate at 37°C for 60 min.
7. Wash plate 3 times with
8. 01M TBS or
9. 01M PBS, and each time let washing buffer stay in the wells for 1 min.
10. Add
11. 1ml of prepared ABC working solution into each well and incubate at 37°C for 30 min.
12. Wash plate 5 times with
13. 01M TBS or
14. 01M PBS, and each time let washing buffer stay in the wells for 1-2 min.
15. Add 90μl of prepared TMB color developing agent into each well and incubate at 37°C in dark for 25-30 min.
16. Add
17. 1ml of prepared TMB stop solution and read OD value at 450nm within 30 min.

## **DATA ANALYSIS**

To analyze using manual methods, follow the process of duplicate readings for standard curve data points and averaging them. Create a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. Calculate the concentration of Klk1 in each sample by interpolating from the standard curve using the average absorbance of each sample.

## **STANDARD CURVE**

Standard curve data:

|  |  |
| --- | --- |
| **Concentration (pg/ml)** | **O.D.** |
| 0 | 0.0 |
| 62.5 | 0.103 |
| 125 | 0.217 |
| 250 | 0.425 |
| 500 | 0.824 |
| 1000 | 1.623 |
| 2000 | 2.243 |
| 4000 | 2.965 |

*This standard curve is for demonstration only. A standard curve must be run with each assay.*

## **REPRODUCIBILITY**

Samples were tested in four different assay lots to assess reproducibility.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Lot 1** | **Lot 2** | **Lot 3** | **Lot 4** | **SD** | **CV** |
| Sample 1 | 258 pg/ml | 265 pg/ml | 262 pg/ml | 260 pg/ml | 3.2 | 1.2% |
| Sample 2 | 1240 pg/ml | 1238 pg/ml | 1252 pg/ml | 1245 pg/ml | 6.5 | 0.5% |
| Sample 3 | 3520 pg/ml | 3480 pg/ml | 3510 pg/ml | 3485 pg/ml | 18.2 | 0.5% |

## **DISCLAIMER**

This material is sold for in-vitro use only in manufacturing and research. This material is not suitable for human use. It is the responsibility of the user to undertake sufficient verification and testing to determine the suitability of each product's application. The statements herein are offered for informational purposes only and are intended to be used solely for your consideration, investigation and verification.