**Mouse KLK1 ELISA Kit**

**Catalog #: IMSKLK1KT** | **Lot #: 20250424**

## **INTENDED USE**

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

## **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.

## **PRINCIPLE OF THE ASSAY**

The Boster Picokine® 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.

## **TECHNICAL DETAILS**

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| **Capture/Detection Antibodies** | The capture antibody is monoclonal antibody from rat and the detection antibody is polyclonal antibody from goat. |
| **Specificity** | Natural and recombinant Mouse Klk1 |
| **Standard Protein** | Expression system for standard: NS0; Immunogen sequence: I25-D261 |
| **Cross-reactivity** | This kit is for the detection of Mouse Klk1. No significant cross-reactivity or interference between Klk1 and its analogs was observed. This claim is limited by existing techniques; therefore, cross- reactivity may exist with untested analogs. |
| **Sensitivity** | N/A |

## **KIT COMPONENTS**

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| **Component** | **Quantity** |
| Anti-Mouse Klk1 Pre-coated 96-well Strip Microplate | 1 |
| Mouse Klk1 Standard | 2 |
| Mouse Klk1 Biotinylated Antibody (100x) | 1 |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 |
| Sample Diluent | 1 |
| Antibody Diluent | 1 |
| Avidin-Biotin-Peroxidase Diluent | 1 |
| Color Developing Reagent (TMB) | 1 |
| Stop Solution | 1 |
| Wash Buffer (25x) | 1 |
| Plate Sealers | 4 |

## **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.

## **ASSAY PROTOCOL**

1. It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information). Prepare all reagents and working standards as directed previously. Remove excess microplate strips from the plate frame and seal and store them in the original packaging. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C). Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 100 µl of the prepared 1x Biotinylated Anti-Mouse Klk1 antibody to each well. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C). Wash the plate 3 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 2 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C). Wash the plate 5 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 4 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.) Add 100 µl of Stop Solution to each well. The color should immediately change to yellow. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm. Assay Protocol Notes Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.