## CATALOG NO: EK1586

## LOT NO: LOT#\_\_\_\_\_\_\_

## INTENDED USE

This Mouse KLK1 Kallikrein 1 ELISA Kit is for research use only. Not for diagnostic procedures.

## BACKGROUND

Kallikrein-1 (KLK1) is a member of the kallikrein subfamily of serine proteases. Kallikreins are involved in post-translational processing of many polypeptides. Kallikrein 1 (KLK1), also known as tissue kallikrein, is one of the 15 known human kallikreins and has a key role in the cardiovascular system. OVERVIEW TECHNICAL DETAILS PREPARATIONS BEFORE ASSAY Please read the following instructions before starting the experiment. This section includes both regular paragraphs and numbered instructions. Before beginning, make sure to thoroughly read the entire protocol.

## PRINCIPLE OF THE ASSAY

This ELISA employs a specific antibody against the target protein coated on a 96-well strip plate. The detection antibody is a biotinylated antibody specific for the target protein. The capture antibody is monoclonal antibody and the detection antibody is polyclonal antibody. To measure the target protein, 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 substrate for HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to the target protein in the sample.

## OVERVIEW

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| --- | --- |
| Product Name | Mouse KLK1 Kallikrein 1 ELISA Kit |
| Reactive Species | No detectable cross-reactivity with other relevant proteins |
| Size | 96T |
| Description | Mouse KLK1 Kallikrein 1 ELISA Kit for Serum, Plasma, Cell Culture Supernatants, Urine |
| Sensitivity | 1 pg/ml |
| Detection Range | 1.56-100 pg/ml |
| Storage Instructions | Store at 4°C for 6 months, -20°C for 12 months |
| Uniprot ID | P15947 |

## TECHNICAL DETAILS

|  |  |
| --- | --- |
| Capture/Detection Antibodies | Rabbit |
| Specificity | Natural and recombinant Mouse KLK1 |
| Standard Protein | Recombinant Mouse KLK1 |
| Cross-reactivity | No detectable cross-reactivity with other relevant proteins |

## PREPARATIONS BEFORE ASSAY

Please read the following instructions before starting the experiment. This section includes both regular paragraphs and numbered instructions. Before beginning, make sure to thoroughly read the entire protocol. Avoid using reagents from different batches together. The kit should not be used beyond the expiration date on the kit label.

1. Prepare all reagents, samples, and standards according to the instructions.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Spin down all components to the bottom of the tube before opening.
4. Don't let the 96-well plate dry out as this will inactivate active components.
5. Don't reuse tips and tubes to avoid cross-contamination.

## KIT COMPONENTS/MATERIALS PROVIDED

|  |  |  |  |
| --- | --- | --- | --- |
| **Description** | **Quantity** | **Volume** | **Storage of opened/reconstituted material** |
| Anti-Mouse KLK1 Precoated 96-well strip microplate | 1 | 96 wells | 4°C |
| Mouse KLK1 Standard | 2 | 10ng/tube | -20°C |
| Anti-Mouse KLK1 Detection Antibody | 1 | 130 μL | -20°C |
| HRP-Streptavidin | 1 | 130 μL | 4°C |
|  |  |  |  |
|  |  |  |  |
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## MATERIALS REQUIRED BUT NOT PROVIDED

* Microplate reader capable of measuring absorbance at 450nm
* Precision pipettes and pipette tips
* Distilled or deionized water

## 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.

## SAMPLE PREPARATION

## DILUTION OF STANDARD

1. Label 7 tubes, one for each standard: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml. 2. Pipette 300 µl of the Sample Diluent into each tube. 3. Pipette 300 µl of the reconstituted standard into the first tube and mix to create the 4000 pg/ml standard. 4. Pipette 300 µl from the 4000 pg/ml tube into the second tube and mix to create the 2000 pg/ml standard. 5. Continue this process for the remaining tubes. 6. The Sample Diluent serves as the zero standard (0 pg/ml).

## TYPICAL DATA / STANDARD CURVE

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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| --- | --- |
| **Concentration (pg/ml)** | **O.D.** |
| 0 | 0.028 |
| 62.5 | 0.061 |
| 125 | 0.143 |
| 250 | 0.227 |
| 500 | 0.405 |
| 1000 | 0.631 |
| 2000 | 1.118 |
| 4000 | 1.902 |

## INTRA/INTER-ASSAY VARIABILITY

- Intra-Assay Precision: Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision: Three samples of known concentration were tested in separate assays to assess inter-assay precision.

## ASSAY PROTOCOL

1. 1. Prepare all reagents, samples, and standards according to the instructions.
2. 2. Confirm that you have the appropriate non-supplied equipment available.
3. 3. Spin down all components to the bottom of the tube before opening.
4. 4. Don't let the 96-well plate dry out as this will inactivate active components.
5. 5. Don't reuse tips and tubes to avoid cross-contamination. Avoid using reagents from different batches together. The kit should not be used beyond the expiration date on the kit label. KIT COMPONENTS MATERIALS REQUIRED BUT NOT PROVIDED Microplate reader capable of measuring absorbance at 450nm Precision pipettes and pipette tips Distilled or deionized water Tubes for standard and sample dilution

## DATA ANALYSIS

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the average zero standard optical density. Plot 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## 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.