**Mouse KLK1/Kallikrein 1 ELISA Kit**

**CATALOG NO**: IMSKLK1KT **LOT NO**: SAMPLE

**INTENDED USE**

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.

**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**

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.

**specification**

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| --- | --- |
| **Sensitivity** | <12 pg/ml |
| **Detection Range** | 62.5 pg/ml - 4,000 pg/ml |
| **Specificity** | Natural and recombinant Mouse Klk1 |
| **Standard** | 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. |

**Reagents**

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| Description | Quantity |
| Pre-coated 96-well strip microplate | 12 strips of 8 wells |
| Standard | 2 x 10ng/tube |
| Biotinylated antibody (100x) | 100 µl |
| Avidin-Biotin-Peroxidase Complex (100x) | 100 µl |
| Sample Diluent | 30ml |
| Antibody Diluent | 12ml |
| Avidin-Biotin-Peroxidase Diluent | 12ml |
| Color Developing Reagent (TMB) | 10ml |
| Stop Solution | 10ml |
| Plate Sealers | 4 |
| Wash Buffer | 20 ml |

**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.

**Typical Data**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration(pg/ml)** | 0.0 | 62.5 | 125 | 250 | 500 | 1000 | 2000 | 4000 |
| **O.D** | 0.028 | 0.061 | 0.143 | 0.227 | 0.405 | 0.631 | 1.118 | 1.902 |

**Typical Standard Curve**

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

A graph of a mouse

AI-generated content may be incorrect.

**INTRA/INTER ASSAY VARIABILITY**

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

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

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| --- | --- | --- | --- | --- | --- | --- |
|  | **Intra-Assay Precision** | | | **Inter-Assay Precision** | | |
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | *16* | *16* | *16* | *24* | *24* | *24* |
| **Mean(pg/ml)** | *150* | *602* | *1476* | *154* | *580* | *1608* |
| **Standard deviation** | *9.15* | *43.94* | *116.6* | *11.08* | *51.62* | *136.68* |
| **CV(%)** | *6.1%* | *7.3%* | *7.9%* | *7.2%* | *8.9%* | *8.5%* |

**Reproducibility**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Lots** | **Lot1 (pg/ml)** | **Lot2 (pg/ml)** | **Lot3 (pg/ml)** | **Lot4 (pg/ml)** | **Mean (pg/ml)** | **Standard Deviation** | **CV (%)** |
| **Sample 1** | 150 | 154 | 170 | 150 | 156 | 8.24 | 5.2% |
| **Sample 2** | 602 | 649 | 645 | 637 | 633 | 18.55 | 2.9% |
| **Sample 3** | 1476 | 1672 | 1722 | 1744 | 1653 | 105.74 | 6.3% |

\*number of samples for each test n=16.

**Procedural Notes**

Please read the following instructions before starting the experiment.

1. 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.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don’t reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

**Reagent Preparation and Storage**

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| Item | Preparation |
| **All reagents** | Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C. |
| **Wash buffer** | Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. |
| **Antibody** | It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Klk1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| **Avidin-Biotin-Peroxidase Complex** | It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin- Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| **Standard** | It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Mouse Klk1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. |
| **Microplate** | The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging. |
| **Samples** | Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Innovative Research’s internal QC testing used:  Dilution ratio of 1:100, concentration in serum and plasma is 50-80 ng/ml. |

**Dilution of standard**

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 4,000.00 pg/ml, 2: 2,000.00 pg/ml, # 3: 1,000.00

pg/ml, # 4: 500.00 pg/ml,

# 5: 250.00 pg/ml, # 6: 125.00 pg/ml, # 7: 62.50 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).

1. To generate standard #1, add 400 µl of the reconstituted standard stock solution of 10 ng/ml and 600 µl of sample diluent to tube #1 for a

final volume of 1000 µl. Mix thoroughly.

1. Add 300 µl of sample diluent to tubes # 2-7.
2. To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.
3. To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix

thoroughly.

1. Continue the serial dilution for tube # 4-7.

A diagram of a test tube

AI-generated content may be incorrect.

**Sample Preparation and Storage**

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| Sample Type | Procedure |
| Cell culture supernatants | Clear sample of particulates by centrifugation, assay immediately, or store samples at -20°C. |
| Serum | Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C. |
| Plasma | Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.  \*Note: it is important to not use anticoagulants other than the ones described above to treat plasma, for other anticoagulants could block the antibody binding site. |
| Cell lysates | Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant. |

**SAMPLE Collection Notes**

1. Innovative Research recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Innovative Research is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

**Sample Dilution Guideline**

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

**Assay Procedure**

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment.

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. 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.
4. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C).
5. 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.
6. Add 100 µl of the prepared **1x Biotinylated Anti-Mouse Klk1 antibody** to each well.
7. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).
8. Wash the plate 3 times with the **1x wash buffer**:
9. 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.
10. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).
11. Repeat steps a-b 2 additional times.
12. 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.
13. 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).
14. Wash the plate 5 times with the **1x wash buffer**:
15. 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.
16. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).
17. Repeat steps a-b 4 additional times.
18. 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.
19. 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.)
20. Add 100 µl of **Stop Solution** to each well. The color should immediately change to yellow.
21. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

**data analysis**

To analyze using manual methods, follow the process below:

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: [www.myassays.com/four-parameter-logistic-curve.assay.](http://www.myassays.com/four-parameter-logistic-curve.assay) Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

**DISCLAIMER**

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