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| --- | --- | --- | --- |
| Description | Quantity | Volume | Storage of opened/reconstituted material |
| 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 |  |

**Mouse KLK1/Kallikrein 1 ELISA Kit (EK1586)**

Catalog Number: EK1586  
Lot Number: SAMPLE

## INTENDED USE

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

## 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

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.

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

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).

PREPARATIONS BEFORE ASSAY

1. Prepare all reagents, samples, and standards according to the instructions.

## TECHNICAL DETAILS

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| --- | --- |
| Capture/Detection Antibodies | 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. |
| Specificity | Natural and recombinant Mouse Klk1 |
| Standard Protein | 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. |
| 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. |

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| Product Name | Mouse KLK1/Kallikrein 1 ELISA Kit |
| Reactive Species | 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. |
| Range | 62.5 pg/ml - 4,000 pg/ml |
| Sensitivity | <12 pg/ml |
| Sample Type | Cell culture media, serum, plasma, and other biological fluids |
| Sample Volume | 100 μl |
| Assay Time | 4.5 hours |
| Protocol | 4.5 hours |

## 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.

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. Avoid using reagents from different batches.

## SAMPLE PREPARATION AND STORAGE

When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation). To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended. Before using the kit, spin tubes to bring down all components to the bottom of the tubes. Don’t let the 96-well plate dry out since this will inactivate active components on the plate. Don’t reuse tips and tubes to avoid cross-contamination. Avoid using the reagents from different batches together.

## SAMPLE COLLECTION NOTES

Innovative Research recommends that samples are used immediately upon preparation. Avoid repeated freeze/thaw cycles for all samples. 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. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.

## SAMPLE DILUTION GUIDELINE

These sample collection instructions and storage conditions are intended as a general guideline. Sample stability has not been evaluated.

### SAMPLE COLLECTION NOTES

Innovative Research recommends that samples are used immediately upon preparation.

Avoid repeated freeze-thaw cycles for all samples.

Samples should be brought to room temperature (18-25°C) before performing the assay.

|  |  |
| --- | --- |
| Sample Type | Collection and Handling |
| Cell Culture Supernatant | Centrifuge at 1000 × g for 10 minutes to remove insoluble particulates. Collect supernatant. |
| Serum | Use a serum separator tube (SST). Allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or store samples at -20°C. |
| Plasma | Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g within 30 minutes of collection. Store samples at -20°C. |
| Cell Lysates | Collect cells and rinse with ice-cold PBS. Homogenize at 1×10^7/ml in PBS with a protease inhibitor cocktail. Freeze/thaw 3 times. Centrifuge at 10,000×g for 10 min at 4°C. Aliquot the supernatant for testing and store at -80°C. |

## SAMPLE DILUTION GUIDELINE

To inspect the validity of experimental operation and the appropriateness of sample dilution proportion, it is recommended to test all plates with the provided samples. Dilute the sample so the expected concentration falls near the middle of the standard curve range.

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.

## TYPICAL DATA / STANDARD CURVE

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

## INTRA/INTER-ASSAY VARIABILITY

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

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

## REPRODUCIBILITY

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

## 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. 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. Background on Klk1 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. 1 Mouse KLK1/Kallikrein 1 ELISA Kit

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Concentration (pg/ml) | 0.028 |  |  |  |  |  |  |  |
| O.D. | 0.061 |  |  |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | n | Mean (pg/ml) | Standard Deviation | CV (%) |
| Sample 1 | 16 | 4.6% | 10.15 | 7.0% |
| Sample 2 | 16 | 5.1% | 11.23 | 7.5% |
| Sample 3 | 16 | 4.8% | 9.88 | 6.7% |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | n | Mean (pg/ml) | Standard Deviation | CV (%) |
| Sample 1 | 24 | 7.8% | 13.05 | 9.0% |
| Sample 2 | 24 | 8.2% | 14.27 | 9.6% |
| Sample 3 | 24 | 8.4% | 12.69 | 8.8% |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Lot 1 | Lot 2 | Lot 3 | Lot 4 | Standard Deviation | Mean |
| Sample 1 | 150 | 154 | 170 | 150 | 156 | 5.2% |
| Sample 2 | 602 | 649 | 645 | 637 | 633 | 2.9% |
| Sample 3 | 1476 | 1672 | 1722 | 1744 | 1654 | 7.2% |