

# PrimePlex Single Plex Kit for Kallikrein 4, KLK4

**REF** : KPXRA450

Ver 1.0

**RUO**

Enzyme Immunoassay for the Quantitative Determination of Kallikrein 4, KLK4 in serum, plasma and other biological samples

|                                                                                     |                              |                                                                                     |                                       |
|-------------------------------------------------------------------------------------|------------------------------|-------------------------------------------------------------------------------------|---------------------------------------|
| <b>RUO</b>                                                                          | <b>For Research Use Only</b> | <b>REF</b>                                                                          | <b>Catalog Number</b>                 |
|  | <b>Store At</b>              | <b>LOT</b>                                                                          | <b>Batch Code</b>                     |
|  | <b>Manufactured By</b>       |  | <b>Biological Risk</b>                |
|  | <b>Expiry Date</b>           |  | <b>Consult Operating Instructions</b> |

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**REF** KPXRA450  96 tests

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**Introduction:**

The PrimePlex Flow Luminescence Immunoassay (FLIA) kits are designed for the quantitative determination of specific biomarkers in biological samples such as serum, plasma, and cell culture supernatants, as validated for each kit. The assay employs a bead-based sandwich Flow Luminescence Immunoassay technique, providing high specificity and enhanced sensitivity. In this format, two highly specific antibodies are utilised: a capture antibody immobilised on fluorescently coded microspheres and a detection antibody that binds to a different epitope of the target analyte. This dual-antibody approach significantly reduces non-specific interference and improves assay performance compared with single-antibody assay formats. The fluorescence signal generated is directly proportional to the concentration of the target analyte present in the sample.

**Intended Use:**

The PrimePlex Single Plex Kit for Kallikrein 4, KLK4 FLIA kit is used as an analytical tool for quantitative determination of Kallikrein 4, KLK4 in serum, plasma and other biological samples.

**Principle:**

The method employs Sandwich FLIA technique. Anti-KLK4 antibodies are pre-coated onto magnetic beads, which serve as the solid-phase carrier. Standards and samples are added to the beads, allowing KLK4 present in the sample to bind specifically to the immobilized capture antibodies. Subsequently, biotinylated anti-KLK4 detection antibodies are added, forming an antibody–antigen–antibody sandwich complex. Following a washing step to remove unbound detection antibodies, PE-labelled avidin is introduced, which binds to the biotinylated antibodies. The resulting fluorescence signal is directly proportional to the concentration of KLK4 in the sample. The Median Fluorescence Intensity (MFI) is measured using a flow cytometer, and the KLK4 concentration in the samples is calculated from the generated standard curve.

**Materials Provided:**

1. Uncoated Microtiter Plate (96 wells) - 1 no
2. Pre-Mix Magnetic bead – 1 ml
3. Setup Bead – 0.4 ml
4. KLK4 Standard (lyophilized, 10 ng/ml) - 2 vials
5. Biotinylated CHAT Antibody (concentrated) - 120 ul
6. Streptavidin:PE Conjugate (concentrated) - 120 ul
7. Standard Diluent - 20 ml
8. Biotin Antibody Dilution Buffer - 12 ml
9. PE Conjugate Dilution Buffer - 12 ml
10. (20X) Wash Buffer - 25 ml
11. TMB Substrate - 12 ml
12. Stop Solution - 12 ml
13. Instruction Manual

**Materials to be provided by the End-User:**

1. Flow Cytometer: NovoCyte D2060R, Navios, FACSMelody, Gallios, DxFLEX, CytoFLEX S, etc. (It is recommended that the instrument be preheated, self-checked and calibrated before use).
2. Single or multi-channel pipettes with high precision and disposable tips.
3. Microcentrifuge Tubes.
4. Deionized or distilled water.
5. Magnetic frame.
6. Container for Wash Solution.
7. 0.01mol/L (or 1×) Phosphate Buffered Saline (PBS), pH 7.0~7.2.
8. Vortex oscillator.
9. Shaker Incubator.

**Handling/Storage:**

1. **For unused kit:** For experiment convenience, reagents should be stored separately, Standard, Standard Diluent, Assay Diluent A and Detection Reagent A should be stored at -20°C while the others could be at 4°C. Pre-Mixed Magnetic beads and Detection Reagent B should be protected from light.
2. **For used kit:** When the kit is used, the remaining reagents need to be stored according to the above storage conditions.

**Note:** It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box. All components are stable up to the expiration date.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
5. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH 7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

**Note:**

- a. Frozen samples should be centrifuged again for 20~30mins at 10,000×g at 2~8°C after thawing, then take the supernatant for assay immediately.
- b. Samples to be used within one week may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- c. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
- d. The lipids, colloids or precipitates in the sample will cause the aggregation and sedimentation of magnetic beads and influence the final detection result. It is recommended to centrifuge the sample first and test the sample supernatant.
- e. Samples should be slowly equalized to room temperature before use, and should not be heated to melt.
- f. Cell cultures with animal serum may contain high levels of latent factor in the preparation of cell culture media and will affect the results.
- g. **When the tissue homogenate sample reacts with magnetic beads, incubate 120mins protect from light at 18~25°C on shake.**

**Sample Dilution**

Please note the kit is validated for use with neat samples.

The user should estimate the concentration of the target protein in the test sample and choose an appropriate dilution factor to ensure that the final concentration falls within the kit's optimal detection range. Dilute the sample using PBS. Multiple trial dilutions may be required to achieve accurate results.

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X) 500 ml**; dilute **20 ml of (30X) Wash Buffer in 580 ml of DI water**.
4. **Streptavidin:PE Conjugate & Biotinylated KLK4 Antibody Working Solution** - Briefly spin or centrifuge the Streptavidin:PE Conjugate & Biotinylated KLK4 Antibody before use. Dilute them to the working concentration 100-fold with PE Conjugate Dilution Buffer & Biotin Antibody Dilution Buffer, respectively.
5. **Standards Preparation**: Reconstitute original Kallikrein 4, KLK4 with 0.5 ml of Standard Diluent. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

| Standard Concentration | Standard Vial | Dilution Particulars                           |
|------------------------|---------------|------------------------------------------------|
| 20 ng/ml               | Standard No.8 | Reconstitute with 0.5 ml Standard Diluent      |
| 5 ng/ml                | Standard No.7 | 125 ul Standard No.8 + 375 ul Standard Diluent |
| 2.5 ng/ml              | Standard No.6 | 250 ul Standard No.7 + 250 ul Standard Diluent |
| 1.25 ng/ml             | Standard No.5 | 250 ul Standard No.6 + 250 ul Standard Diluent |
| 0.312 ng/ml            | Standard No.4 | 250 ul Standard No.5 + 250 ul Standard Diluent |
| 0.078ng/ml             | Standard No.3 | 250 ul Standard No.4 + 250 ul Standard Diluent |
| 0.0195 ng/ml           | Standard No.2 | 250 ul Standard No.3 + 250 ul Standard Diluent |
| 0 ng/ml                | Standard No.1 | 250 ul Standard Diluent only                   |

**Procedural Notes:**

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Kallikrein 4 KLK4. High Dose Hook Effect is due to excess of antibody for very high concentrations of Kallikrein 4 KLK4 present in the sample.
3. Kallikrein 4, KLK4 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide ( $\text{NaN}_3$ ), as it could destroy the PE activity resulting in under-estimation of the amount of Kallikrein 4 KLK4.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

**Assay Procedure:**

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Add **200 ul analysis buffer** solution to each well of the plate for pre-wetting. Shake the plate at room temperature for 10mins on shaker. Then, remove the liquid from the well.
3. Add **100 ul of Standard and Samples** in designated wells.
4. Add **10 ul** evenly suspended **magnetic beads** in each well. Cover with the Plate sealer.
5. Cover the plate with a sealer and incubate for 90 minutes at 37°C on a shaker incubator at 800rpm, protected from light. amplitude of 2~4 mm.  
*(Note: When the tissue homogenate sample reacts with magnetic beads, incubate 120mins protect from light at 18~25°C on shake).*
7. Put the plate on magnetic frame for 2 mins, remove the liquid of each well. DO NOT WASH.

8. Remove the magnetic frame, Add **100 ul** of **Biotinylated KLK4 Antibody Working Solution** in each well.
9. Cover the plate with a sealer and incubate for 60 minutes at 37°C on a shaker incubator at 800rpm, protected from light. amplitude of 2~4 mm.
10. Put the plate on magnetic frame for 2 mins, remove the liquid of each well. Add 200 ul of 1× Wash Solution to each well and let it sit for 1~2mins. Remove the liquid of each well with plate on the magnetic frame. Totally wash 3 times. This process could be performed by magnetic plate autowasher.
11. Remove the magnetic frame, Add **100 ul Streptavidin:PE Conjugate** to each wells.
12. Cover the plate with a sealer and incubate for 30 minutes at 37°C on a shaker incubator at 800rpm, protected from light. amplitude of 2~4 mm.
13. Put the plate on magnetic frame for 2 mins, remove the liquid of each well. Add 200 ul of 1× Wash Solution to each well and let it sit for 1~2mins. Remove the liquid of each well with plate on the magnetic frame. Totally wash 3 times. This process could be performed by magnetic plate autowasher.
14. Remove the magnetic frame, add 100 ul of sheath fluid to each well, cover the wells with the plate sealer and protect from light, shake the plate at 37°C for 10mins on shaker with speed of 800rpm, amplitude of 2~4mm. Make the Magnetic beads in suspension, then run the machine and conduct the reading.

#### Instrument settings:

##### A.

| NovoCyte D2060R                     |                  |
|-------------------------------------|------------------|
| Analytes volume                     | 50 ul            |
| Fluorescent                         | APC, APC-Cy7, PE |
| Total Event                         | 100*N            |
| Median Fluorescence Intensity (MFI) | Median           |

**N = the number of targets detected by this kit.**

##### B.

| Luminex 200,FLEXMAP 3D              |               |
|-------------------------------------|---------------|
| Analytes volume                     | 70 ul         |
| Bead Type                           | MagPlex       |
| Total Event                         | 50/region     |
| Median Fluorescence Intensity (MFI) | Median        |
| DDGate                              | 7500 to 15000 |

#### Calculation of Results:

This assay employs the competitive inhibition immunoassay technique, so there is an inverse correlation between KLK4 concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log graph paper, with the log of KLK4 concentration on the y-axis and the log of MFI on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, for instance, curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

#### Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:  
 - If the sample absorbance value is below the first standard.

#### Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

#### Performance Characteristics of the Kit:

This kit has been validated. Please view the details herein below.

**Standard Calibration Range:**

0.0195 ng/ml – 20 ng/ml.

**Sensitivity:**

**Limit Of Quantification:**

It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.0065 ng/ml.

**Safety Precautions:**

- **This kit is For Research Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
  - Do not smoke, eat or drink while handling kit material.
  - Always use protective gloves.
  - Never pipette material by mouth.
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



**Typical Example of a Work List**

| Well #   | Contents                       | Absorbance at 450nm | Mean Absorbance | Interpolated Concentration |
|----------|--------------------------------|---------------------|-----------------|----------------------------|
| 1A<br>2A | Standard No.1<br>Standard No.1 |                     |                 |                            |
| 1B<br>2B | Standard No.2<br>Standard No.2 |                     |                 |                            |
| 1C<br>2C | Standard No.3<br>Standard No.3 |                     |                 |                            |
| 1D<br>2D | Standard No.4<br>Standard No.4 |                     |                 |                            |
| 1E<br>2E | Standard No.5<br>Standard No.5 |                     |                 |                            |
| 1F<br>2F | Standard No.6<br>Standard No.6 |                     |                 |                            |
| 1G<br>2G | Standard No.7<br>Standard No.7 |                     |                 |                            |
| 1H<br>2H | Standard No.8<br>Standard No.8 |                     |                 |                            |
| 3A<br>4A | Sample<br>Sample               |                     |                 |                            |
| 3B<br>4B | Sample<br>Sample               |                     |                 |                            |

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