NOVATEINBIO

Accelerates Research and Development

Porcine Kidney Injury Molecule 1,

KIM1 ELISA Kit

Catalog number: BG-POR11442 (96 wells)

The kit is designed to detect the level of Porcine KIM1

in cell culture supernatant, serum, plasma and other suitable sample solution

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; After reading the subsequent contents of this manual, please note the following specially :

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standard, testing samples are tested in duplicate.
- Use serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time (Do not over develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- Chromogenic Solution B is light-sensitive. Avoid prolonged exposure to the light.

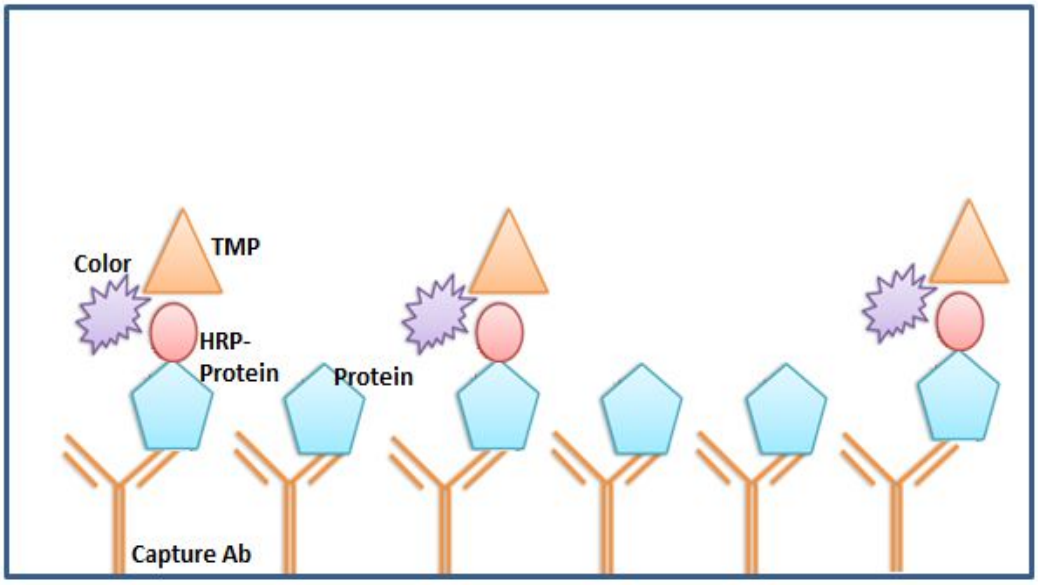
Intended use

The kit is used to quantify the Porcine KIM1 in cell culture supernatant, serum, plasma and other suitable sample solution. This assay has high sensitivity and excellent specificity for detection of Porcine KIM1. No significant cross-reactivity or interference between KIM1 and analogues was observed.

Standard range	0.5 - 10 ng/ml
Sensitivity	0.1 ng/ml
Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

The Porcine KIM1 ELISA kit applies a competitive enzyme immunoassay technique utilizing a polyclonal anti-Porcine KIM1 antibody and a Porcine KIM1 conjugate. The assay sample and buffer are incubated together with KIM1-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the KIM1 concentration since KIM1 from samples and KIM1-HRP conjugate compete for the anti-KIM1 antibody binding site. Since the number of sites is limited, as more sites are occupied by KIM1 from the sample, fewer sites are left to bind KIM1-HRP conjugate. Standards of known KIM1 concentration are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density) to the concentration of KIM1. The KIM1 concentration in each sample is interpolated from this standard curve.



Materials supplied

1	Microelisa Stripplate	96 well
2	Standard	1.0 ml X 6 vials
3	100 X Wash Solution	10 ml
4	Balance Solution	3 ml
5	HRP-Conjugate Reagent	6 ml
6	Chromogenic Solution A	6 ml
7	Chromogenic Solution B	6 ml
8	Stop Solution	6 ml
9	Package insert	1

Note: Standard (S1 - S6) concentration was followed by: 0, 0.5, 1.0,2.5, 5.0, 10 ng/ml

Materials required but not supplied

- 37℃ incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.
- **Cell culture fluid and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

  NOTE: The Balance Solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; if the sample is serum or blood plasma, then the Balance Solution is a superfluous reagent. Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤1months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Sample Preparation

- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degratation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- Dispense 10 µl of Balance Solution into 100µl specimens, mix and stand for one hour (The proportion of Balance Solution and Specimens should be no less than 1:10).
  (NOTE: This step is required when the samples are cell culture fluid & tissue homogenate, if the sample is serum or blood plasma, then this step should be skipped.)
- **Wash Solution** -Dilute 10 mL of Wash Solution concentrate (100 ×) with 990 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1 ×).

Assay procedures

1. Prepare all reagents before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.
2. Secure the desired numbers of coated wells in the holder. Take the Standards (S1-S6) and agitate gently prior to use then add 100µl Standards following the sequence of S1 to S6 to the appropriate well of the antibody pre-coated Microtiter Plate.
3. Add 100 µl saline to the blank wells. Add 100 µl Samples to the appropriate well. Generally, you should get the sample value within the assay arrange without dilution. If samples generate values higher than the highest standard, further dilute the samples with 0.1%BSA in PBS, pH7.4 (without Mg^{2+} & Ca^{2+}) and repeat the assay.

4. Add 50 µl of Conjugate to each well (except the blank wells). Mix well. **Mixing well in this step is important.** Cover and incubate the plate for 1 hour at 37°C.
5. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

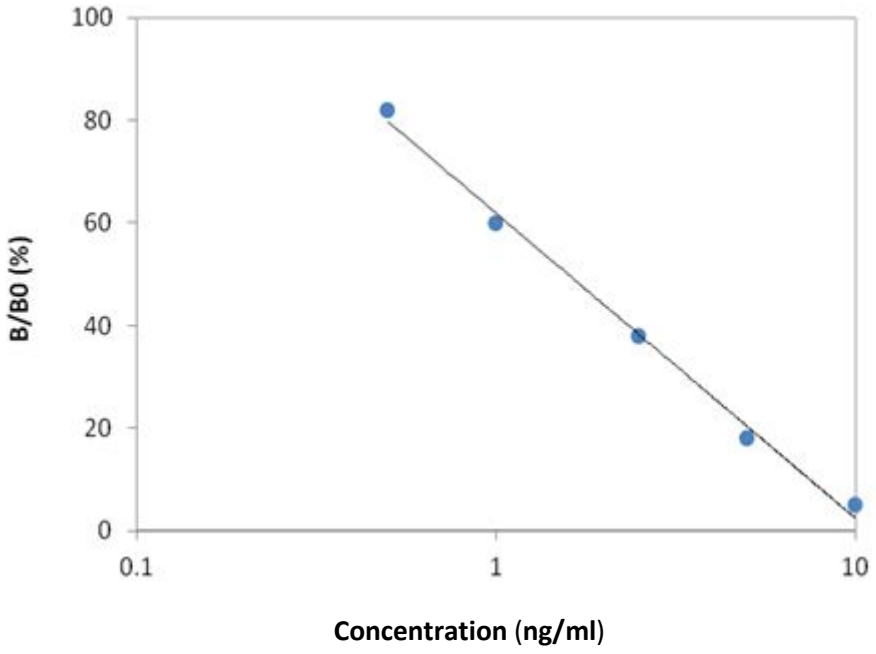
Automated Washing: Wash plate FIVE times with diluted wash solution(350-400µl/ well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

6. Add 50 µl Chromogenic Substrate A and 50µl Chromogenic Substrate B to each well, subsequently. Cover and incubate for 10 minutes at 20-25°C. (Protect from light).
7. Add 50 µl Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

- The standard curve is used to determine the amount of samples.
- First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the blank control before result interpretation.
- Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve e using graph paper or statistical software to generate a four paramater logistic (4-PL) curve-fit or logit-log linear regression curve. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. A standard curve should be generated for each assay.

Typical data:



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