



User's Manual

# Pig coagulation factor VIII ELISA Kit



DEIASL525



96T





This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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## PRODUCT INFORMATION

### Intended Use

For the quantitative determination of pig coagulation factor VIII (F8) concentrations in serum, plasma, tissue homogenates.

### Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for F8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any F8 present is bound by the immobilized antibody. After removing any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for F8 is added to the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of F8 bound in the initial step. The color development is stopped and the intensity of the color is measured.

### Reagents And Materials Provided

1. Assay plate: 1(96 wells)
2. Standard (Freeze dried): 2
3. HRP-conjugate (100 x concentrate): 1 x 120  $\mu$ l
4. HRP-conjugate Diluent: 1 x 20 ml
5. Sample Diluent: 2 x 20 ml
6. Wash Buffer (25 x concentrate): 1 x 20 ml
7. TMB Substrate: 1 x 10 ml
8. Stop Solution: 1 x 10 ml
9. Adhesive Strip (For 96 wells): 4
10. Instruction manual: 1

### Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. An incubator which can provide stable incubation conditions up to 37°C $\pm$ 0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100ml and 500ml graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

## Storage

1. The unopened kit should be stored at 2 - 8°C. Do not use the kit beyond the expiration date.
2. Coated assay plate may be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp.
3. Standard and HRP-conjugate may be stored for up to 1 month at 2 - 8°C. If don't make recent use, better keep it stored at -20°C.
4. The other reagents may be stored for up to 1 month at 2 - 8°C.

## Specimen Collection And Preparation

### Serum

Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 xg. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

### Plasma

Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 xg at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

### Tissue Homogenates

100 mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 xg, 2 - 8°C. The supernate was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

### SAMPLE DILUTION

Serum and plasma samples require a 10-fold dilution into Sample Diluent. The suggested 10-fold dilution can be achieved by adding 25µl sample to 225µl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

### Note:

1. Creative Diagnostics 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 samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. 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.

6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. 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.
8. 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.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

## Reagent Preparation

Note:

1. Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
2. Bring all reagents to room temperature (18-25°C) before use for 30min.
3. Prepare fresh standard for each assay. Use within 4 hours and discard after use.
4. Making serial dilution in the wells directly is not permitted.
5. Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize

imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.

6. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. HRP-conjugate (1x) - Centrifuge the vial before opening.

HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP-conjugate Diluent.

2. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

3. Standard

Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 4000 µg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

## Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells

and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.

3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 60 minutes at 37°C. A plate layout is provided to record standards and samples assayed.
4. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100µl of HRP-conjugate (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 60 minutes at 37°C.
6. Repeat the aspiration/wash process for five times as in step 4.
7. Add 90µl of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light.
8. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Samples may require dilution. Please refer to Sample Preparation section.

## Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.

As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the F8 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Precision

Intra-assay Precision (Precision within an assay): CV%<8%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

## Detection Range

62.5 ng/mL-4000 ng/mL

## Sensitivity

15.62 ng/mL

## Specificity

This assay has high sensitivity and excellent specificity for detection of pig F8. No significant cross-reactivity or interference between pig F8 and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between pig F8 and all the analogues, therefore, cross reaction may still exist.

## Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## Limitations

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.
5. Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.