Porcine IL-8 ELISA kit

Catalog No.E0080p

(96 tests)

Operating instruction

www.eiaab.com

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Intended use

This immunoassay kit allows for the specific measurement of total porcine interleukin 8 (IL-8) concentrations in cell culture supernates, serum , plasma, BALF and other specimen.

Introduction

Interleukin 8 (IL-8), a member of the neutrophil-specific CXC subfamily of chemokines, is a potent neutrophil chemotactic and activating factor. It is a primary inflammatory cytokine produced by many cells (including monocytes/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, astrocytes and chondrocytes) in response to proinflammatory stimuli such as IL-1, TNF, LPS and viruses. Its function is, in part, to attract neutrophils to the site of inflammation and to activate them.

The IL-8 cDNA sequence predicts a protein of 99 amino acids. Removal of a 22-residue signal peptide generates a mature protein of 77 amino acids (~ 8 kDa). Further proteolysis of the N-terminal end leads to a variant form with 72 amino acids; full activation of IL-8 may require cleavage to the 72 amino acid form. IL-8 can form non-covalent dimers in solution, especially at high concentrations, but dimerization is not necessary for biological activity.

IL-8 binds to two seven-transmembrane, G protein-coupled receptors, CXCR1 and CXCR2, as well as to the non-signalling Duffy antigen on red-blood cells. The Duffy antigen may play a role in regulating IL-8 activity on functional receptors.

Test principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-8 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-8 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 x 20ml
Assay Diluent A	1 x 10ml
Assay Diluent B	1 x 10ml
Detection Reagent A	1×120ul
Detection Reagent B	1×120ul
Wash Buffer (25 x concentrate)	1 x 30ml
Substrate	1 x 10ml
Stop Solution	1 x 10ml

Limitations of the procedure

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

Sample collection and storage

Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

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

Note: Hemolyzed samples are not suitable for measurement of porcine IL-8 with this assay.

Reagent preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

IL-8 Standard -Reconstitute Standard with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high

standard (1000 pg/mL). The **Sample Diluent** serves as the zero standard (0 pg/mL).
Detection Reagent A and B - Dilute to the working concentration specified on the vial label using **Assay Diluent A and B** (1:100), respectively.

Assay procedure

Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Add 100 uL of **Standard**, Control, or sample* per well. Cover with the adhesive strip. Incubate for 2 hours at 37° C.
3. Remove the liquid of each well, don't wash.
4. Add 100 uL of **Detection Reagent A** to each well. Incubate for 1 hours at 37° C . **Detection Reagent A** may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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.
6. Add 100 uL of **Detection Reagent B** to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37° C.
7. Repeat the aspiration/wash as in step 5.
8. Add 90 uL of **Substrate Solution** to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Calculation of results

Average the duplicate readings for each standard, control, 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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-8 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.

Sensitivity

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of IL-8 ranged from 0.12 - 0.97 pg/mL. The mean MDD was 0.28 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Precaution

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