Human Pseudomonas Exotoxin A, PEA ELISA kit

Catalog No.E0974h

(96 tests)

Operating instruction

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 for the in vitro quantitative determination of human Pseudomonas Exotoxin A, PEA concentrations in cell culture supernates, serum, plasma and other biological fluids.

Introduction

Exotoxin A of Pseudomonas aeruginosa is a secreted bacterial toxin capable of translocating a catalytic domain into mammalian cells and inhibiting protein synthesis by the ADP-ribosylation of cellular elongation factor 2. The protein is a single polypeptide chain of 613 amino acids. The x-ray crystallographic structure of exotoxin A, determined to 3.0- angstrom resolution, shows the following: an amino-terminal domain, composed primarily of antiparallel ß -structure and comprising approximately half of the molecule; a middle domain composed of -helices; and a carboxyl-terminal domain comprising approximately one-third of the molecule. The carboxyl-terminal domain is the ADP-ribosyltransferase of the toxin. The other two domains are presumably involved in cell receptor binding and membrane translocation.

Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to PEA. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for PEA and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain PEA, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of PEA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and components

Reagent	**Quantity**
Assay plate	1
Standard	2
Sample Diluent	1 x 20ml

Assay Diluent A	1 x 10ml
Assay DiluentB	1 x 10ml
Detection Reagent A	1 x 120ul
Detection Reagent B	1 x 120ul
Wash Buffer (25 x concentrate)	1 x 30ml
Substrate	1 x 10ml
Stop Solution	1 x 10ml

Sample collection and storage

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 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 30 minutes of collection. Store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.

Cell culture supernates 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: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8 ° C, otherwise samples must stored at -20° C (≤ 3 months) or -80° C (≤ 6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.

It is recommended that all samples be assayed in duplicate.

DO NOT USE HEAT-TREATED SPECIMENS.

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 soluble receptors, 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.

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 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Standard - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution

produces a stock solution of 20,000 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 (20,000 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. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100 uL of **Standard**, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37° C.
2. Remove the liquid of each well, don't wash.
3. Add 100 uL of **Detection Reagent A** working solution to each well. Incubate for 1 hour at 37°C. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. 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.
5. Add 100 uL of **Detection Reagent B** working solution to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37° C.
6. Repeat the aspiration/wash as in step 5.
7. Add 90 uL of **Substrate Solution** to each well. Incubate for 30 minutes at 37°C. Protect from light.
8. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and natural human PEA. No significant cross-reactivity or interference was observed.

Sensitivity

The minimum detectable dose of human PEA is typically less than 78 pg/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

Detection Range

312-20,000 pg/mL. The standard curve concentrations used for the ELISA's were 20,000 pg/mL, 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312 pg/mL.

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.
7. Do not substitute reagents from one kit lot to another. Standard, conjugate a microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

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 PEA 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.

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 (six months 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. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
4. 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.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Substrate Solution is easily contaminated. If bluish prior to use, do not use.

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.