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User's Manual

Cefquinome Elisa Kit

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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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Cefquinome ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of cefquinome in milk.

General Description

Cefquinome is a fourth-generation cephalosporin with pharmacological and antibacterial properties valuable in the treatment of coliform mastitis and other infections. It is only used in veterinary applications.

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The cefquinome antibody has been coated in the plate wells. During analysis, sample is added along with the cefquinome-horseradish peroxidase (cefquinome-HRP) conjugate. If the cefquinome residue is present in the sample, it will compete for the cefquinome antibody, thereby preventing the cefquinome-HRP from binding to the antibody attached to the well. The resulting color intensity, after addition of the HRP substrate (TMB), has an inverse relationship with the cefquinome residue concentration in the sample.

Reagents And Materials Provided

Coated plate

Standards: 6

Enzyme conjugate

Milk extraction buffer

Milk balance buffer

Wash solution

Substrate

Manual

Materials Required But Not Supplied

Microtiter plate reader (450 nm)

Vortex mixer (e.g. Genie Vortex mixer from VWR)

10, 20, 100 and 1000 µL pipettes

Multi-channel pipette: 50-300 µL (Optional)

Storage

Store the kit at 2 - 8°C.

Specimen Collection And Preparation

1. Add 1.5 mL of milk sample into a plastic tube. Add 150 µL of PLM Milk Extraction Buffer to each sample.
2. Vortex vigorously for 30 seconds.
3. Centrifuge at 4,000 × g for 10 minutes.
4. Take 660 µL of supernatant and add 200 µL of PLM Milk Balance Buffer, mix well by vortexing.
5. Use 100 µL of the sample for the assay.

Assay Procedure

1. Add 100 µL of each Cefquinome Standard in duplicate into different wells (Add standards to plate only in order from low concentration to high concentration).
2. Add 100 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of Cefquinome-HRP Conjugate and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 - 25°C) in the dark.
5. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash the plate 3 times with 250 µL of 1× Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100 µL of Substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating. Incubate for 20 minutes at room temperature (20 - 25°C) in the dark. (To avoid potential contamination, do not put any substrate back into the original container. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended.)
7. After incubation, add 100 µL of Stop Buffer to stop the enzyme reaction.
8. Read the plate as soon as possible following the addition of Stop Buffer, on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings.)

Calculation

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 / absorbance zero standard

Specificity

Cefquinome 100%

Precautions

Do not use the kit past the expiration date.

Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC.

Try to maintain a laboratory temperature of 20 - 25°C. Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.

Make sure you are using only distilled or deionized water since water quality is very important.

When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.

Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.

Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 - 25°C) while in the packaging.