Version 20-04/24

User's Manual

3-methyl-quinosaline-2·carboxylicacid ELISA Kit

REF **DEIANJ14**

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RUO

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

The 3-methyl-quinosaline-2·carboxylicacid(MQCA) ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of MQCA in chicken, duck and other sample types.

General Description

MQCA (3-methyl-quinoxaline-2-carboxylic acid) is a metabolite of N-(2-hydroxyethyl)-3-methyl-2-quinoxaline-carboxamide-1,4-dioxide (Olaquindox). Olaquindox is a broad spectrum antibiotic that is used in animal feed for growth promotion, to improve feed efficiency and to increase the rate of weight gain. In 1991, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended maximum residue limits (MRLs) of 30 and 5 μg/kg in liver and muscle tissues, respectively. In 1998, the European Commission (EC) banned the use of olaquindox in food animal production together with four other feed additives. In 2005, it was banned for use as a veterinary drug by Chinese government regulatory agencies.

Principles of Testing

The method is based on a competitive colorimetric ELISA assay. The plate wells are coated with anti-MQCA antibody. During analysis, sample is added along with the MQCA-horseradish peroxidase (MQCA-HRP) conjugate. If MQCA residue is present in the sample, it will compete for the MQCA antibody, thereby preventing the MQCA-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 concentration of MQCA residue in the sample.

Reagents And Materials Provided

MQCA Coated Plate 1 × 96-well Plate (8 wells x 12 strips)

MQCA Standards:

Negative control (white cap tube) 1.8 mL

0. 8 ng/mL (yellow cap tube) 1.8 mL

2. 0 ng/mL (orange cap tube) 1.8 mL

5 ng/mL (pink cap tube) 1.8 mL

12 ng/mL (purple cap tube) 1.8 mL

30 ng/mL (blue cap tube) 1.8 mL

Spiking 500 ng/mL(red cap tube) 1.8 mL

MQCA-HRP Conjugate 6 mL

20× Wash Solution 28 mL

1× Meat Extraction Buffer 25 mL

10× Sample Extraction Buffer 25 mL

Stop Buffer 14 mL

TMB Substrate 12 mL

Materials Required But Not Supplied

Microtiter plate reader (450 nm primary filter, 630 nm differential filter)

Tissue Mixer (e.g. Omni Tissue Master Homogenizer)

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

10, 20, 100 and 1000 µL pipettes

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

Distilled deionized water

Disposable plastic reservoirs

Paper towels

Ethyl Acetate

Storage

Store the kit at 2 - 8°C.

Specimen Collection And Preparation

Be sure samples are stored properly. Samples should be refrigerated at 2 - 4°C for no more than 1 - 2 days. Freeze samples to a minimum of - 20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temperature (20 - 25°C) or in a refrigerator before use.

1. Preparation of 1× Sample Extraction Buffer

Mix 1 volume of 10× Sample Extraction Buffer with 9 volumes of distilled water.

Chicken/Duck/Pork

1. Homogenize a reasonable amount of sample with a suitable mixer.
2. To 3 g of a homogenized sample in a tube, add 6 mL of ethyl acetate, 2 mL of distilled water, and 200 uL 1X Meat Extraction Buffer. Manually vortex for 3 minutes at maximum speed, or vortex for 10 minutes using a multi-tube vortexer.
3. Add 1 mL of 2 M sulfuric acid. Vortex for 30 seconds at maximum speed.
4. Centrifuge for 5 minutes at 4,000 × g at room temperature (20 - 25°C).
5. Slowly transfer 2 mL of the ethyl acetate supernatant (corresponding to 1 g of the original sample) into a new vial and use a rotary evaporator to dry the sample in a 60 - 70°C water bath under reduced pressure. Alternatively, the sample can be dried by blowing nitrogen gas over it in a 60 - 70°C water bath.
6. To the dried residue, add 1 mL of hexane and 1 mL of 1× Sample Extraction Buffer. Vortex at maximum speed for 1 minute.
7. Centrifuge for 5 minutes at 4,000 × g at room temperature (20 - 25°C).
8. Remove the top hexane layer completely. Use 100 µL of the bottom layer in each well for the assay.

Note: Dilution factor = 1.

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 - 2 hours at 20 - 25°C); Make sure you read the Warnings and Precautions section.

Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination, and is recommended.

1. Preparation of MQCA-HRP

Add 4 mL of HRP Diluent to one tube of MQCA-HRP Conjugate powder. Gently invert the tube for 10 times and incubate the solution at room temperature for at least 30 minutes. Then invert the tube 5 times immediately before usage. The prepared MQCA-HRP can be stored at 4°C for one week or -20°C freezer for one month.

2. Preparation of 1× Wash Solution

Mix 1 volume of 20× Wash Buffer with 19 volumes of distilled water.

Assay Procedure

1. Add 100 µL of each MQCA 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 MQCA-HRP Conjugate and mix well by rocking the plate manually against a hard force point 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 tap the plate dry on paper towels. Perform the next step immediately after plate washings. Do not allow the plate to completely air dry.
6. Add 100 µL of TMB substrate. Incubate the plate for 15 minutes at room temperature (20 - 25°C) in the dark. Time the reaction immediately after adding the substrate. Mix the solution by rocking the plate manually against a hard force point for 1 minute while incubating. 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.

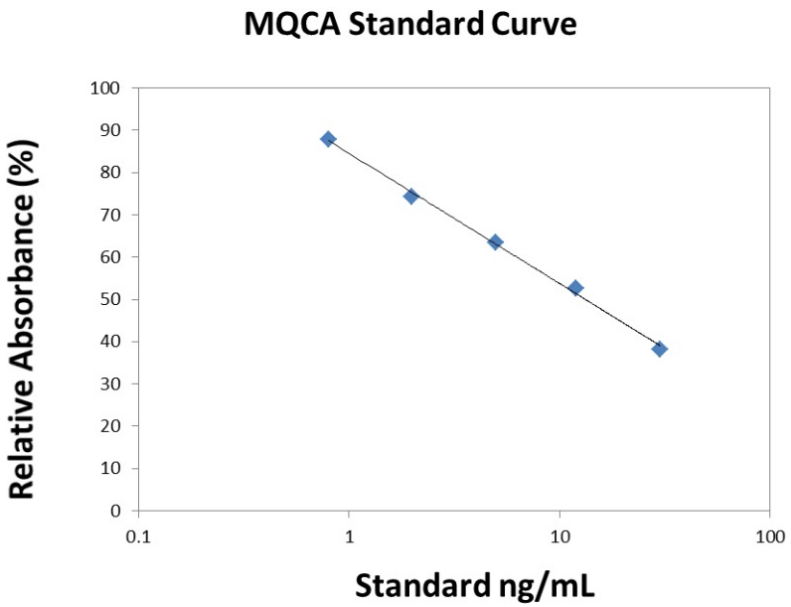
Calculation

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

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve.

Typical Standard Curve



Precision

Interplate and intraplate CV typically < 10%

Detection Limit

Chicken 1 ppb

Duck 1 ppb

Pork 1 ppb

Sensitivity

0.5 ppb

Specificity

MQCA 100%

Precautions

The standards contain MQCA. Handle with particular care.

Do not use the kit past the expiration date.

Do not mix reagents from different kits or lots, except for components with the same part number within their expiration dates. HRP-CONJUGATES 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 other insulation material under the assay plates during incubation.

Make sure you are using only distilled or deionized water; 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 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.