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Nitrofurantoin (AHD) ELISA kit

Catalogue Number:SL0030Ot

1 Use purpose

This kit for Feed, fish, shrimp and meat tissue (such as chicken, beef and pork), eggs, honey, milk, serum and urine in the AHD remaining quantitative detection.

2 Experimental principle

This kit adopts methods in competition ELISA In microplates coated with antigen conjugated AHD, add AHD standard or samples, Free AHD and pre-coated on strips of AHD conjugated antigen compete against AHD antibody conjugates, With TMB chromogenic substrate,the color is changed from blue to yellow after adding stop solution, enzyme standard instrument 450nm wavelengths in testing, absorb light value and in the sample was AHD content is inversely proportional to the standard curve, through calculation samples was AHD concentrations.

3 Materials provided with the kit

3.1 Microelisa stripplate: 1 block (12well×8strips).
3.2 AHD standard: six vials of (1ml/ vial), content is respectively: 0 PPB, 0.1 PPB, 0.3 PPB and 0.9 PPB, 2.7 PPB, 8.1 PPB.
3.3 Anti- AHD antibody conjugate: 1 vial (6ml).
3.4 Chromogen Solution A: 1 vial (6ml).
3.5 Chromogen Solution B: 1 vial (6ml).
3.6 Stop Solution: 1 vial (6ml), 2M sulphuric acid.
3.7 sample dilution: 1 vial (10 x, 6ml), used for sample diluted with.
3.8 wash solution: 1 vial (20 x, 20ml), used for washing board.
3.9 Instruction.

4 Need not provide materials

4.1 equipment
4.4.1 wavelength 450nm microplate.
4.1.2 shredder.
4.1.3 LiangTong.
4.1.4 oscillators.

4.1.5 funnel.
4.1.6 Whatman No 1 or equivalent filter paper.
4.1.7 trace remove liquid device.
4.2 reagent
4.2.1 the deionized water or distilled water.
4.2.2 methanol.

5 Storage

5.1 kit stored in 2 ~ 8 ℃, Don't frozen
5.2 Don't use up the Microelisa stripplate should be sealed drying preserve

6. Precautions

6.1 please read the instructions carefully , before useing the kit.
6.2 don't use expired kit.
6.3 before using the kit, please let the reagent recover to room temperature (25 + 2 ℃), the proposal for at least 2 hours to temperature.
6.4 the standard contain AHD, pay special attention to, the operation, we should bring gloves.
6.5 stop solution is containing sulfate, when using, prevent burns skin and corrosion clothing.
6.6 different standard and sample suction head used cannot be mixed use, otherwise, it will affect test results.
6.7 different batches of reagent kit not mix, Different standard and sample suction head shall not be used in combination, otherwise, it will affect the experimental result.
6.8 diluted sample must use this kit of sample diluent, otherwise, it will affect the experimental result
6.9 mixed reagents should avoid blistering.

7 Working liquid preparation

7.1Carbendazim standards: 0ppb, 0.1 PPB, 0.3 PPB and 0.9 PPB, 2.7 PPB, 8.1 PPB
7.2 wash solution: 1:20 with distilled water (1 +19) diluted. preparation
7.3 sample dilutions: 1:10 with distilled water (1 +9) diluted. preparation
7.3 Chromogen Solution reagent: already standby, avoid light straight as
7.4 stop solution: already termination aside

8 Sample processing program (sample in extraction process, must strictly according to the operation of the extraction process should be accurate dilution, can

appear otherwise results are inaccurate, samples should be kept in a cool place to avoid light and frozen keep)
8.1 Smash the samples taken 10g, add 20ml 70% methanol solution
8.2 powerful oscillation 3 minutes
8.3 Whatman No 1 filter with
8.4 take 100 μl treatment of samples, join 400 μl samples diluent
8.5 take 25 μl treatment of samples, add 25 μl samples dilutions in reaction well (sample diluted times for 2)

9 Enzyme 2linded analysis steps

9.1 experimental guidelines
9.1.1 experiment begins prior to all reagent in boxes outside the room temperature (25 fully recovered to + 2 ℃), time about 2 hours. Return to room temperature (25 + 2 ℃) again after remove Strips, excess pore bar to seal immediately to the 2 ~ 8 ℃ drying preserve
Note: be sure to temperature, otherwise fully guarantee the accuracy and precision of the affecting detection.
9.1.2 after use please immediately reagent put back 2 ~ 8 ℃ preservation
9.1.3 please don't change analysis program
9.1.4 please use accurate trace remove liquid device
9.1.5 operation once started, please do not interrupt any program
9.1.6 ELISA results of repeatability of severe depends on operating procedures, please strictly according to requirements operation
9.1.7 to avoid cross-contamination, each standard and samples should use different suction head add samples
9.1.8 plus sample do make suck a head contact microporous the solution or inside surface
9.2 analysis steps
9.2.1 beforehand numbered, mark B0, standard and the sample position recommended double orifice detection
9.2.2 take the required amount of the Strips (Strips detachable), will spare attrib wattle and sealed immediately put back again 2 ~ 8 ℃ preservation
9.2.3 sample dilutions (10), wash solution x (20 x) dilution into working liquid (distilled water or deionized water dilute)
In 9.2.4 B0 well joining 50μl 0.0 ng/ml standard
9.2.5 in each standard well joining 50μl standard
9.2.6 in each sample well joining 50μl sample solution
9.2.7 In all well joining 50 μl Anti- AHD antibody conjugate
9.2.8 gently sloshing response board for a few seconds.

9.3 37 ℃ warm bath 30min (warm bath process sometimes pat reaction plate, can reduce double orifice error)
9.3.1 Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat..
9.4 reaction
9.4.1 washing procedure completed, immediately with liquid apparatus in every trace move microporous first join 50 μl Chromogen Solution A, add 50μl Chromogen Solution B, Slight sloshing response board make thoroughly incorporated
37 9.4.2 ℃ warm bath 10min
9.4.3 each well joining 50 μl Stop Solution, blending
9.4.4 in 450nm testing absorbency, result in 5min inside read.

10 The results calculated

10.1 quantitative analysis
10.1.1 obtained by each concentration standard solution and the average value of a sample spectrophotometry (B) divided by the first standard (0 standard absorbency value (B0) multiplying by 100%, namely percentage absorbency values.
B - standard solution or sample solution of average absorbance of the values
B0-0 μg/L standard solution of average absorbance of the values
10.1.2 with AHD concentrations of values for the X axis, 100 cent absorbance of the value of the Y axis, draw standard curve. According to the sample percentage absorbency values, which get corresponding points from curve, namely the abscissa denotes the multi-goal AHD concentration on the numerical curvature.the against several namely to determine AHD concentration C (ppb)
10.1.3 because the sample after diluted in advance, so according to the standard curve gains from different concentration samples must again multiply the diluted times.
10.2 half quantitatively
10.1.1 visual half quantitative determination: first, choose an appropriate standard fluid and samples with operation, according to the samples and standard substance absorbency value the discretion of the judge is compared, sample chroma value is less than or greater than standard values.
10.1.2 instrument half quantitative determination: first, choose an appropriate standard fluid and samples with operation, according to the sample and standard color depth comparison, judge sample chroma value is less than or greater than standard values.

11 Specificity

Physical cross reaction

AHD 100%

12 Kit parameters

This kit detection limit is 0.05 PPB
B0 absorbance of the optimal value should be greater than 1.0
Kit absorbency board inside error is less than 8%, board between error is less than 15%.
With this manual provided a tissue sample extraction method recovery is greater than 80%.

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Kit provides standard curve scope for 0.1 PPB ~ 8.1 PPB.

14 Analysis restriction

This kit testing positive for samples should use another method such as HPLC or GC/MS to be verified.