

## Colistin (Col) ELISA Kit

**Catalog No.:** abx365004

**Size:** 96T

**Storage:** Store at 4°C for up to 6 months. Once opened, store at 4°C and use within a month.

**Application:** For quantitative detection of Colistin in muscle tissue and urine.

**Sensitivity:** 1 ppb (ng/ml)

**Detection Limit:** Muscle Tissue – 20 ppb, Urine – 10 ppb

**Cross-reactivity:** Colistin – 100%, Polymyxin B – 165%

### Principle of the Assay

This kit is based on competitive enzyme-linked immuno-sorbent assay technology. The well plate is pre-coated with coupled antigen. The standards, samples and antibody solution are added to the wells and incubated. After washing away the unbound conjugates, HRP-conjugate is added to each microplate well. After TMB substrate solution is added, only wells that contain Colistin will produce a blue colour product that changes into yellow after adding the stop solution. The intensity of the yellow colour is inversely proportional to the Colistin amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of Colistin can be calculated.

<b>Kit components</b>	<b>Materials Required But Not Provided</b>	<b>Reagents Required But Not Provided</b>
1. One pre-coated 96 well plate	1. 25°C incubator	1. Anhydrous methanol
2. Standards - 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb, 1 ml each	2. Microplate reader (450 nm)	2. Deionized water
3. HRP Conjugate reagent: 12 ml	3. High-precision pipette and sterile pipette tips	
4. Antibody Solution: 10 ml	4. Automated plate washer	
5. Substrate Reagent A: 6 ml	5. ELISA shaker	
6. Substrate Reagent B: 6 ml	6. 50 ml centrifuge tubes	
7. Stop Solution: 6 ml	7. Absorbent filter papers	
8. Wash Buffer (20X): 25 ml	8. 100 ml and 1 L graduated cylinders	
9. Sample Solution (20X): 40 ml	9. Homogenizer	
10. Sample Diluent: 50 ml	10. Centrifuge	
11. Plate sealer: 3 pieces		
12. Hermetic bag: 1		

## Protocol

### A. Preparation of sample and reagents

#### 1. Preparation of Sample pretreatment solutions

- **Solution 1 – Sample Solution Preparation**

Dilute the 20X Sample Solution 20-fold with 5% methanol solution - i.e. add 40 ml of 20X Sample Solution to 760 ml 5% methanol solution to make the 1X Sample Solution.

- **Solution 2 – Wash Buffer**

Dilute the 20X Wash Buffer 20-fold with deionized water - i.e. add 25 ml of 20X Wash Buffer to 475 ml deionized water to make the 1X Wash Buffer solution.

#### 2. Sample pretreatment

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Muscle Tissue (*Chicken, Duck, and Pig samples*):** Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Weigh 1 g of homogenised muscle tissue and add to a 50 ml centrifuge tube. Add 4 ml of 1X Sample Solution, and vortex for 5 mins. Centrifuge the homogenates at 4000 rpm for 5 mins at room temperature, and then collect 200 µL of supernatant. Transfer the supernatant to a new tube and then add 200 µL of Sample Diluent. Vortex for 5 minutes. Aliquot 20 µl and assay immediately. **Note: Sample dilution factor: 10, minimum detection dose: 20 ppb.**
- **Urine (*Pig samples*):** Centrifuge the urine sample until clear. Collect 50 µl of centrifuged urine sample and add 450 µl of Sample Diluent. Vortex for 1 min. Aliquot 20 µl and assay immediately. **Note: Sample dilution factor: 10, minimum detection dose: 10 ppb.**

**Note:**

- » Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
- » Please bring sample slowly to room temperature.

## B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate or triplicate. Add the solution at the bottom of each well without touching the side walls. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles.
2. Add 20 µl of the standard solutions into the standard wells.
3. Add 20 µl of prepared sample into test sample wells.
4. Immediately add 80 µl of Antibody solution to each well (including control well). Add the solution at the bottom of each well without touching the side wall.. Measure the absorbance at 450 nm immediately.
5. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 25°C for 15 mins.
6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (260 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Add 100 µl of HRP Conjugate Reagent into each well (including control well).
8. Cover the plate with the plate sealer. Tap the plate gently to ensure thorough mixing. Incubate in the dark at 25°C for 15 mins.
9. Repeat the wash step as described in step 6.
10. Add 50 µl of Substrate Reagent A and 50 µl of Substrate Reagent B into each well (including control well). Tap the plate gently to ensure thorough mixing. Cover the plate and incubate in the dark at 25°C 15 mins. Please note that the incubation time is for reference only - do not exceed 30 minutes. When an apparent gradient appears in the standard wells the reaction can be terminated.
11. Add 50 µl of Stop Solution into each well (including the blank well). Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between Colistin concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**Note:** If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

## C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. Do not use components from a different kit or expired ones.
10. Substrate Reagent A and Substrate Reagent B are easily contaminated; work under sterile conditions when handling these solutions. The substrate solutions should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.