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Product no AS20 4396

# Tylosin | ELISA quantitation kit

## Background

This ELISA assay utilizes the principle of competitive binding to measure the concentration of Tylosin in analyzed samples. The Tylosin antigen has been pre-coated on the surface of the reaction wells. Samples, containing an unknown amount of Tylosin, or standards are added to the appropriate plate wells together with specific anti-Tylosin antibodies and Horseradish Peroxidase (HRP) conjugated anti-antibody. During incubation, a competitive inhibition reaction occurs between the pre-coated Tylosin and the Tylosin in the standards and the analyzed samples with anti-Tylosin antibody. Substrate solution is added to the wells and the color develops in opposite to the amount of Tylosin in the sample or standards. The reaction is stopped, and the intensity of the color is measured at 450 nm.

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## The Tylosin ELISA kit reagents

| Reagent | Amount |
|---|---|
| Assay plate | 1 (96 wells) |
| Standard | 6 x 1 ml |
| HRP-conjugate | 1 x 7 ml |
| Antibody | 1 x 7 ml |
| Substrate A | 1 x 7 ml |
| Substrate B | 1 x 7 ml |
| Stop Solution | 1 x 7 ml |
| Sample Diluent (2x concentrate) | 1 x 50 ml |
| Wash Buffer (20x concentrate) | 1 x 40 ml |

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**Sensitivity:** <1.5 ppb
**Detection range:** 1.5 ppb-121.5 ppb
**Recovery rate:** Honey ± 18%, Liver 80% ± 22%, Muscle 80% ± 25%,
**Limit of detection:** Honey: 1.5 ppm, Liver and Muscle: 3 ppm,
**Sample volume:** 50 µl
**Detection wavelength:** 450 nm
**Assay development time:** 1-2 h
**Intra-assay precision (within assay):** CV%<10%
**Inter-assay precision (between assays):** CV%<10%
**Storage:** 2-8°C
**Cross-reactivity:** Tylosin 100%
**Sample type:** Honey, Liver, Muscle

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### Not provided in the kit but required to conduct the test

Microplate reader capable of measuring absorbance at 450 nm, (correction wavelength set at 540 nm or 570 nm) **|** Incubator with stable incubation temperature up to 25°C **|** Squirt bottle, manifold dispenser or automated microplate washer **|** Centrifuge, vortex mixer **|** Rotary evaporator or nitrogen gas **|** Analytical balance, 2 decimal place **|** Single-channel micropipette (20-200 µl, 100-1000 µl) **|** Multi-channel micropipette (30-300 µl) **|** 100 ml and 500 ml graduated cylinders **|** Absorbent paper for blotting the microtiter plate **|** Deionized or distilled water **|** Pipette and pipette tips **|** Test tubes for dilution **|** Methanol **|** NaOH **|** Concentrated HCl **|** Trichloromethane **|** Acetonitrile

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### Important notes

The kit should not be used beyond the expiration date on the kit label. Reagents from different lots should not be mixed. In case analyzed samples are generating values which are higher than the highest standard, samples should be diluted, and the assay repeated. The assay may vary due to factors such as operator, pipetting technique, washing technique, incubation time, temperature, and kit expiry date. This assay is designed to eliminate the interference caused by soluble receptors, binding proteins, and other components present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

**Samples:** Samples should be added very carefully to avoid foaming and touching the well wall. For each step in the procedure, the total dispensing time for the addition of reagents or samples to the assay plate should not exceed 10 min, to ensure equal time elapsed for each pipetting step. Cross contamination should be avoided by changing pipette tips between additions of each standard, sample and reagent.

**Incubation:** Proper adhesion of plate sealers during each incubation step must be applied to ensure accurate results. Strips should not be let dry at any time. Incubation temperature and time must be kept constant with the optimal temperature being 37°C.

**Washing:** This step is critical, and the complete removal of liquid at each step is essential for a good performance of this assay. Any drops and fingerprints must be removed from a bottom of the plate. Insufficient washing will result in poor precision and false absorbance readings. In case of automated washing, addition of a 30 s soak period, following the addition of wash buffer and plate rotation by 180 degrees between each wash step, may improve assay precision.

**Reaction development:** Substrate should change from colorless or light blue to gradations of blue. In case the color is too intense, Stop Solution should be added in advance. This will avoid inaccurate absorbance readings due to an excessively strong reaction. Substrate solution can be easily contaminated. The liquid should remain colorless or light blue until added to the plate. Keep this solution away from any light.

**Stop solution:** Stop Solution should be added to the plates in the same order as the Substrates. The reaction is developed correctly if color development in the wells goes from blue to yellow upon addition of the Stop Solution. Green color indicates that the Stop Solution was not thoroughly mixed with the substrate.

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### Safety measures

The Tylosin quantitation kit Stop Solution is an acidic solution. Please use suitable protective gear for your eyes, hands, face, and clothes when handling this item.

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### Procedure instructions

Please read the whole manual carefully before proceeding with your experiment.

## Before starting

As a recommendation, all samples and standards should be made in duplicates for this assay. Prepared samples should be analyzed promptly. Do not exceed handling time of 10 minutes per step. Always use a plate cover during all steps. Do not let the strips dry at any time during the assay.

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## Reagent preparation

**0.1 M HCl**
Add 0.86 ml of concentrated HCl to 100 ml deionized or distilled water and mix well.

**0.1 M NaOH**
Add 0.4 g of NaOH to 100 ml deionized or distilled water and mix well.

**(Acetonitrile-0.1 M HCl )-Methanol Mixed Solution**
Mix 84 ml of Acetonitrile and 16 ml of 0.1 M HCl well. Add 18 ml of Methanol and mix well.

**Sample Diluent (1x)**
Mix equal parts Sample Diluent (2x) and deionized or distilled water well.

**Wash Buffer (1x)**
In case crystals have formed, warm the concentrate to room temperature and mix gently until the crystals have dissolved completely. Prepare 400 ml of Wash Buffer (1x) by diluting 20 ml of Wash Buffer Concentrate (20x) with 380 ml deionized or distilled water.

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## Sample preparation

**Honey**
Put 1.00 ± 0.05 g of sample into a 50 ml centrifugal tube. Add 2 ml of deionized water and vortex for 2 min. Add 10 ml of Trichloromethane, up-and-down shock for 2 min. Centrifuge at room temperature for 10 min at a minimum of 4000 rpm. Discard the upper layer of the centrifuged supernatant mix, and use the lower organic layer. The sample can be dried using nitrogen gas. Add 1 ml of Sample Diluent (1x) and shake well for 30 s. Use 50 µl of the sample for further analysis. Dilution factor of the samples: 1.

**Muscle, Liver**
Put 2.00 ± 0.05 g of homogenized sample into a 50 ml centrifugal tube and add 8 ml (Acetonitrile-0.1 M HCl)-Methanol Mixed Solution. Vortex for 2 min, and subsequently centrifuge at room temperature for 10 min at a minimum of 4000 rpm. Move 2 ml of the supernatant into a clean centrifugal tube and add 1 ml of 0.1 M NaOH. Mix well. Add 3 ml of Trichloromethane and vortex strongly for 1 min. Centrifuge at room temperature for 10 min at a minimum of 4000 rpm. Discard the upper layer of the centrifuged supernatant mix, and use the lower organic layer. The sample can be dried using nitrogen gas. Add 1 ml of Sample Diluent (1x) and shake well for 30 s. Use 50 µl of the sample for further analysis. Dilution factor of the samples: 2.

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## Manual

1. Bring all reagents and samples to room temperature before use (keep in RT for at least 30 min).
2. Centrifuge the samples after thawing. All samples and standards should be assayed in duplicates.
3. Prepare the reagents and samples according to sections Reagent Preparation and Sample Preparation.
4. Centrifuge the standard vials at 6000-10000 rpm for 30 s. The most concentrated standard, S5, is at 121.5 ppb, while the zero standard, S0, is at 0 ppb.

| Tube | S0 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|
| ppb | 0 | 1.5 | 4.5 | 13.5 | 40.5 | 121.5 |

5. Determine the number of wells to be used. Unused wells should be put back into the Ziplock pouch and stored at 4°C.
6. Set one blank well without any solution. Add 50 µl of standard or sample to each well. Add samples gently to avoid foaming and be careful not to touch the well walls. Add 50 µl of HRP-conjugate and 50 µl of Antibody to each well. Incubate the microtiter plate at 37°C for 40 min, covered by an unused adhesive strip.
7. Aspirate the wells and wash the plate by filling the wells with Wash Buffer (1x, 250 µl in each well) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto-washer. Let it stand for 15-30 s, remove the liquid completely, and repeat the washing process four times for a total of five washes. After the fifth wash, remove all liquid and invert the plate against clean paper towels. Remove water and fingerprint on the bottom of the plate to avoid falsely reading results. The wash procedure is critical. Complete removal of liquid at each step is essential.
8. Add 50 µl of Substrate A and 50 µl of Substrate B to each well (except the blank well). Mix well and incubate at 37°C for 20 min. Protected from light.
9. Add 50 µl of Stop Solution to each well in the same order as the substrates and mix thoroughly by gently tapping the plate. The color will change from blue to yellow. Green color indicates that the Stop Solution has not mixed well with the Substrates.
10. Read the optical density of each well within 5 minutes, at 450 nm.

| **Standard** | **ppb** | **Average** |
|---|---|---|
| S0 | 0 | 2.243 |
| S1 | 1.5 | 1.816 |
| S2 | 4.5 | 1.415 |
| S3 | 13.5 | 0.740 |
| S4 | 40.5 | 0.313 |
| S5 | 121.5 | 0.155 |

Example of Tylosin standard curve. Note that developed color is inversely proportional to the amount of Tylosin in the sample.

**Results**

There are two methods to judge the results: the first one (A) is the rough judgment, while the second (B) is the quantitative determination.

Note that the OD value of the sample has a negative correlation with Tylosin in the sample.

A: Compare the sample average absorbance values with standards values, the Tylosin concentration in the samples can be concluded. For example, the absorbance value of sample 1 is 0.3, the absorbance value of sample 2 is 1.0; absorbance values of standard are: 2.243, 1.816, 1.415, 0.74, 0.313 and 0.155, and the corresponding concentrations are: 0 ppb, 1.5 ppb, 4.5 ppb, 13.5 ppb, 40.5 ppb and 121.5 ppb, then the Tylosin in sample 1 and sample 2 are 40.5-121.5 ppb and 4.5 ppb-13.5 ppb. Lastly, the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

B: The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$Absorbency\ value\ (\%) = \frac{B}{B_0} \times 100\%$$

$$B: the\ average\ absorbance\ value\ of\ the\ sample\ or\ standard$$
$$B_0: the\ average\ absorbance\ value\ of\ the\ 0\ ppb\ standard$$

To draw a standard curve, take the absorbency value of the standards as y-axis, and the semi-logarithmic of the concentration of the Tylosin standards solution (ppb) as x-axis. The Tylosin concentration of each sample (ppb), read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

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**Related products**

Tylosin, Serum (0.1 ml)

**AS11 1641** | Clonality: Polyclonal | Host: Rabbit | Reactivity: Tylosin