Symmetric Tetracyclines in Honey

Lateral Flow Test Kit

for the detection of tetracyclines in honey

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A.

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

Symmetric Tetracyclines in Honey, S0124, is a lateral flow test kit for the detection of tetracyclines in honey. This kit contains all reagents required for 24 reactions.

Matrices:

Honey

- Test time: 10min
- Shelf life: 12 months
- Storage: 2-8°C

1. Description

Symmetric Tetracyclines in Honey is a Lateral Flow test for the detection of tetracyclines in honey samples.

2. General information

Tetracyclines, a broad-spectrum family of antibiotics, are used for the treatment of a wide variety of bacterial infections, including the American and European foulbrood in bee colonies. Tetracyclines can accumulate in beehives and migrate from the hives to honey leading to product contamination. Consuming products containing tetracycline residues poses a substantial risk, as it can potentially trigger allergic reactions, carcinogenicity, mutagenicity, hepatoxicity and the development of antibiotic resistance. Although the use of antibiotics in apiculture is not authorized in the EU, there are no established Maximum Residue Limits (MRLs) for antibiotics in honey under the European legislation. Hence, there is a significant demand for a rapid and highly sensitive technique to detect tetracyclines in honey.

3. Principle of the method

The Symmetric Tetracyclines in Honey lateral flow test is based on the competitive format immunoassay principle. The wells of the microtiter strips contain tetracycline specific antibodies conjugated to colloidal gold. The diluted honey sample is added into the well. A dipstick with two capture lines, test and control, is dipped into the suspended mixture. If the sample is free of tetracyclines, a color development occurs at the test line, indicating the absence of tetracycline residues in the sample. On the contrary, the presence of tetracyclines in the sample will cause a reduced colored signal at the test line. A valid test should always have the upper control line red.

4. Reagents provided

Symmetric Tetracyclines in Honey kit contains sufficient reagents and materials for 24 measurements.

- 3 containers each with 1 strip of 8 reagent microwells and 8 dipsticks
- 24 sample extraction tubes
- 2 x 50ml extraction solution
- Instruction manual

5. Materials required but not provided

- Balance with 0 - 50g measuring capability
- 100 or 200μL adjustable single channel micropipettes with disposable tips
- Vortex mixer
- Clock or timer
- S-Flow software along with matching scanner device provided by lateral logic ltd

6. Storage instructions

Store kit components between 2 - 8°C. Do not freeze any components provided. Reseal the unused strips in the storing tube together with the desiccant bag provided. The expiry date of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated due to prior handling. Do not interchange individual components between kits of different lot numbers.

7. Safety and precautions for use

All reagents should be brought to room temperature (21 - 25°C) before use (at least half an hour) and covered when not in use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross contamina-

8. Sensitivity

Table 1. Limits of Detection for every compound.

	Compound	LOD (μg/kg)
Tetracyclines	Tetracycline	1 - 3
	Oxytetracycline	1 - 3
	Chlorotetracycline	1 - 3
	Doxycycline	2 - 4

9. Sample preparation

A. Weigh 1.0±0.05g honey sample into a sample extraction tube provided.
B. Add 3mL of the extraction solution.
C. Close the tube and vortex for 3 minutes.
D. Allow to settle for 10 minutes.

10. Method procedure

1. Before opening the reagents, take the kit out of the fridge (at least for half an hour) and wait until the temperature of the reagents reaches the ambient temperature.
2. Open one plastic pot and take out as many tests (dipsticks) and microwells as the number of honey samples to be tested. If needed, using scissors, carefully cut the number of reaction wells.
3. The pot with dipsticks should always be well closed after reagents have been taken out. A pot with dipsticks should be emptied before another is opened.
4. Dispense **100μL** of the prepared diluted honey sample into the microwell. Using the same pipette tip, aspirate the sample up and down about 10 times to completely mix the lyophilized gold particles in the sample, while avoiding bubbles. The sample should turn into a uniform pink color. After mixing the particles, remove and discard the pipet tip.
5. Immediately, place the dipstick into the well and set timer for 10 minutes.
6. When the 10 minutes are over, take the dipstick out of the microwell and remove the white cotton sample-pad of the stick immediately. Touch the stick with your hand from the colorful pad and remove the white pad with your hands. Do not use a paper towel or any other material.
7. Place the dipstick inside the plastic holder in order to be scanned. In case of S-Flow or 3PR scanner, the sticks must be facing up. In case of EPSON scanner, the sticks must be facing down (inverted) and the colored side must be facing the orange sticker.
8. Use S-flow software to interpret results as soon as possible and no later than 1 minute after the end of analysis. The software will use the ratio, R, of the test line and the control line to calculate the results (Table 2).

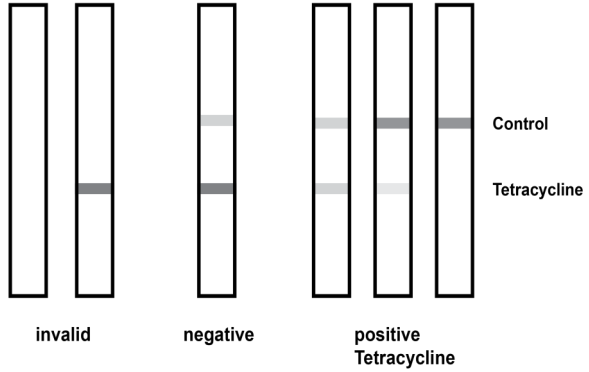
Table 2. Instrumental Interpretation

Ratio	R>1.1	0.9≤R≤1.1	R<0.9
Interpretation	Negative	Weak Positive	Positive

11. Visual interpretation

When the test time is completed (10 min), the dipstick can also be visually read and interpreted according to the following figure.

Visual result interpretation index



1. The control line should always be visible, if not the test is invalid.
2. When the control line can be seen, compare the intensity of test line with the intensity of the control line:

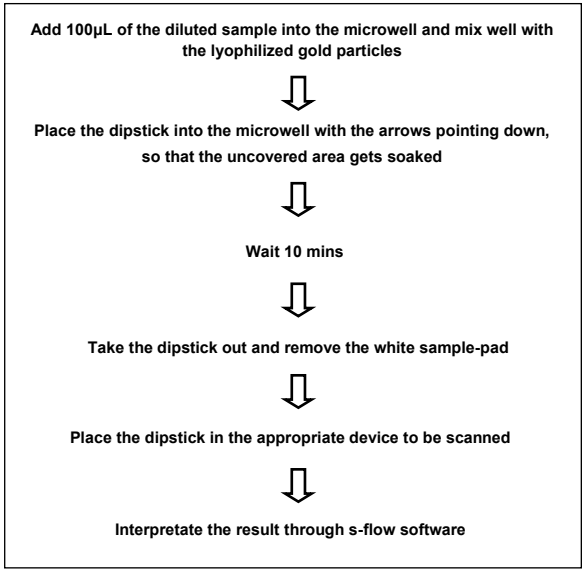
If the test line is darker than the control line (T>C), the sample contains no tetracyclines or tetracyclines at a lower level than the detection limits.

If the test line is the same with the control line (T=C), the sample contains tetracyclines close to the detection limits

If the test line is lighter than the control line (T<C), the sample contains tetracyclines above the detection limits.

12. Method summary

Total method time: 10 minutes.



VERSION N1

CAT.NUMBER: S0124
STORAGE: 2-8°C

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LATERAL FLOW TEST KIT

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