

## Premi®Test sample pre-treatment procedure for honey

### Background

DSM is aware of the problems of antibiotic residues in honey. Several state laboratories are examining honey with the Premitest. DSM did additional tests in which the suitability of PremiTest for detecting antibiotic residues in honey was examined. This protocol is made with information received from customers (e.g. Central Science Laboratory in York (UK) as well as from the internal validation.

#### Protocol for honey without extraction

- Heat the honey at 45°C for 30 minutes.
- Dilute the honey with water (1:1).
- Honey may have a very low pH (around 3), which influences the result of the PremiTest. So adjust the pH to 5.5 – 6.0.
- Adjustment can be done with NaOH or with an acetate buffer (not internal validated!)
- Transfer 100 µl of this mixture onto the agar in the ampoule.
- Depending on the origin of the honey incubation can be done directly at 64°C or with the 2-Step Incubator (first 10 minutes at 80°C).
- Be aware of the dilution: detection level of the Premi®Test increases!

#### Protocol for honey with the extraction method

- Take 2 gram of honey.
- Add 5 ml of acetonitrile / acetone (70:30 v/v)
- Homogenize for 30 - 40 seconds
- Sonicate for 5 minutes, than vortex mix for 30 –40 seconds.

- Centrifuge at 4500 rpm for 10 minutes at 4°C.
- Remove supernatant and evaporate under nitrogen at 40-45°C until approximately 100 µl remains.
- Resuspend residue into 250 µl 8 gram/liter Lab Lemco broth (Oxoid: Cat No CM0015) and mix well.
- Apply 100 µl of this mixture onto the Premi®Test.

### Sensitivity for honey with rapid extraction method

The Premi®Test is validated for **honey** using the acetone extraction method by CSL in the UK. The detection limits obtained were as follow:

Penicillin G:	< 12.5 ppb
Amoxicillin:	< 12.5 ppb
Ampicillin:	25 ppb
Oxacillin:	75 ppb
Cloxacillin:	100 ppb
Dicloxacillin:	< 75 ppb
Sulphadiazine:	75 ppb
Sulphamethizole:	75 ppb
Oxytetracycline:	75 ppb
Chlortetracycline:	80 ppb
Tetracycline:	50 ppb
Doxycycline:	50 ppb

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