

Premi[®]Test sample pre-treatment procedure for poultry- and pig-feed

Background

Using animal feed in a microbial inhibition test may result in false positive results due to the presence of several inhibitors in the raw materials of feed.

To prevent these inhibitors interfering with the test result a special protocol is required to use the Premi[®]Test for the detection of antibiotic residues in poultry- and pig-feed. DSM has developed a proprietary technology. This patented technology is described in this technical bulletin.

Inactivating the proteinaceous factors through a new sample pre-treatment of the poultry- and pig-feed can prevent the influence of naturally occurring inhibiting substances.

Sample preparation method

- Wash hands before use and make sure to use a clean working surface.
- Grind the feed in a mortar.
- Take 10 gram of the grinded feed.
- Add 30 ml of water.
- Stir for half an hour at room temperature.

Instructions for using Premi[®]Test

- Remove the aluminum foil carefully from the ampoule(s).
- Use a clean tip on the syringe for each sample.
- Cut of a small part of the tip-end.
- Transfer 100 µl of the suspension onto the agar in the ampoule(s), by pressing the syringe once and releasing it. It will automatically take up the required volume.
- Close the ampoule(s) with the plastic foil supplied with the kit.
- Check temperature of the 2-step Incubator: 64°C (± 0,5 °C).
- Place the ampoule(s) in the Premi[®]Test 2-Step Incubator and push the "Start/Stop" button (see instructions).

- The Premi[®]Test 2-Step Incubator automatically heats the samples for 10 minutes at 80°C.
- After this heat pre-treatment, the Premi[®]Test 2-Step Incubator automatically incubates the samples at the required temperature of 64°C (± 0,5 °C).
- Incubate the sample until the negative control changes color.
- Use a negative sample (feed of the same origin without antibiotics) as control.

Reading the test results

- When the negative control changes color from purple to yellow, the results can be red.
- Read the results from the bottom 2/3 part of the ampoule
- A clear color change purple to yellow indicates that the antimicrobial compounds are below the Premi[®]Test detection limits.
- A purple color indicates the presence of antibacterial substances at or above the detection limits of the Premi[®]Test.

Premi[®]Test indicative data on detection levels for feed (µg/l):

Group	Substance	Feed (pigs)	Feed (poultry)
β-lactams	Penicillin G	10	10
Macrolides	Tylosin	400	200
Tetracyclines	Oxytetracycline	2000	800
Sulphonamides	Sulphadiazine	>4000	4000
Aminoglycosides	Streptomycin	16000	8000
Ionophores	Salinomycin	20000	8000
Ionophores	Monensin	>8000	6000
Oligosaccharides	Avilamycin	>20000	>20000
Other	Bacitracin	20000	10000

The data indicated in the table are based upon readings judged as being yellow/purple to purple and are to be regarded as an indication. It is recommended to always confirm positive test results. Variations in incubation times and temperatures, operators and storage conditions may have an influence on the reading result found.

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DSM Nutritional Products

DSM Premi[®]Test B.V., P.O. Box 1163,
6160 BD Geleen, The Netherlands. Tel. +31 (0)46 4763573
Fax. +31 (0)46 4763737, Internet site: www.premitest.com

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