

# Premi®Test

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All you need  
is this Starterkit



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# Tips & Tricks for Premi®Test

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## Prepare the ampoule

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**Step 1:**  
Cut off the  
required  
number of  
ampoules.

### Notes:

- Take care not to damage the covering foil of the remaining ampoules.
- Always use a negative control, of the same matrix, in addition to the samples to be tested.
- Regularly (e.g. once a month) use a positive standard in addition to the samples to be tested.
- In such cases use two extra ampoules.

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## Get the meat juice!

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### Step 2:

Take approx. 2  
cm<sup>3</sup> of lean meat.  
Extract about 250  
µl of meat juice  
using the meat  
press delivered  
with the Starterkit  
Premi®Test.



### Multipress:

Solution for squeezing 12 meat samples at once.

### Notes:

- Heat the meat press in an oven or under warm tap water before usage.
- After pressing for a while, turn the meat press upside down.
- This technique works best with fresh red meat!
- For frozen meat, chicken meat or fish: read on for recommended procedure.
- **DO NOT USE MINCED OR GROUND MEAT!!**
- Clean, rinse and dry the meat press after each usage.

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## Juice in the ampoule

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**Step 3:**  
Pipette 100 µl of  
juice slowly onto  
the agar in the  
ampoule.

### Notes:

- Use the syringe delivered with the ampoules; its capacity is exactly 100 µl.
- Take care **NOT TO TOUCH** the AGAR!
- Use a new pipet tip for each sample! Thirty pipet tips are delivered with each PremiTest box, which contains 25 ampoules.
- Make sure that the pipette tip is not blocked by small pieces of meat in the juice! 100 µl is needed to get the right sensitivity.

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## Prediffusion

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### Step 4:

Allow to stand at  
room temperature  
for 20 minutes for  
prediffusion.

### Notes:

- "Room temperature" lies between 15 and 25 °C. Do not allow the samples to stand at temperatures outside this range.
- Plug in the PremiTest Incubator right now, so it can preheat to 64 °C.

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## Get the juice out of the ampoule 6

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### Step 5:

Flush the meat juice out of the ampoule by gently washing twice with demineralized water. Carefully drain the water from the ampoule.



#### Notes:

- Do not try to remove the water from the ampoule by tapping; if agar comes loose from the ampoules this will give false positive results.
- Fill the ampoule with water and turn it upside down to drain the water. Repeat this and then place the vial upside down on a piece of paper so that any remaining drops of water at the rim will be absorbed.

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## Close the ampoule 7

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### Step 6:

Close the test ampoule with foil supplied with the Premi®Test to avoid evaporation.



#### Notes:

- It is essential to use the foil delivered with the Premi®Test!
- This foil has specific characteristics: tiny holes in it prevent water of condensation getting into the ampoule during heating. If you do not use this foil, you could get false positive results.

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## Start the incubation 8

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### Step 7:

Check the temperature of the incubator (64°C). Place the ampoule in the incubator.



#### Note:

- Did you remember to plug in the incubator at the pre-diffusion stage? (see slide 5)

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## Remove the ampoule 9

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### Step 8:

Withdraw the ampoules from the incubator after approx. 3 hours. Determine the color of the lower 2/3 part of the solid agar.



#### Notes:

- At the moment the negative control of one species changes into yellow, read all the samples of the same species!
- If all samples are still purple or suspected, leave them in the incubator for another 5 minutes.
- After these 5 minutes, read again the color of the bottom part of the contents of the ampoules.
- Repeat this operation until the negative control changes color!

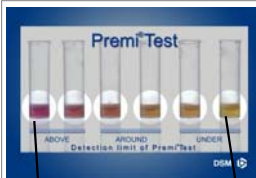
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## Determine the color visually 10

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No color change = no multiplication of Bacillus = presence of an antibiotic

Change of color = multiplication of Bacillus = absence of an antibiotic

#### Notes:

- Determine the color of the lower two thirds of the contents of the ampoule.
- A purple border can appear at the top of the contents.
- Be careful; color reading errors can lead to major problems in interpreting the results.

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## Use Premi®Scan for easy reading 11

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#### Operating System:

- Windows 98 or higher
- CD-ROM drive
- Recommended scanner: HP Scanjet 7400 or 8200 series
- 20MB of free hard drive space, 32MB of internal memory (RAM)
- Q60 Calibration Card and Target File
- Black cloth & templates:

#### Notes:

- The Premi®Scan is particularly useful when reading large series



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## Additional information / FAQ (read these before implementing the Premi®Test!)

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## What samples to take?

Samples should be representative (this depends on the type of animal), but economic considerations also play a role. It would be a waste to use the ham from a pig when the diaphragm is sufficient.

Do not take samples from muscles that are too fatty or too tender.

Examples of suitable samples:

Pork : diaphragm

Beef : lean meat

Poultry : filet

### Notes:

Do not use :

- The tongue
- The kidney, except when kidney is to be tested (in that case use the adapted protocol for kidney)
- The liver, except when liver is to be tested (in that case use the adapted protocol for liver)

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## How much sample?

The minimum volume of a sample is 2 cm<sup>3</sup>.

Generally a bigger volume is taken and frozen in a freezer. A part can be cut off for analysis.

In some cases it will be useful to take two samples and freeze them separately.

### Notes:

- Never refreeze defrosted samples!
- Prevent surface contact between different samples.

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## How many samples per batch?

If the animals were given antibiotics collectively at the farm (e.g. through the feed in the case of broilers or pigs), 2 to 5 samples/animals per batch can be taken. The heterogeneity within a group of animals is predictable, but animal-to-animal variation is not. With 5 samples per batch, the risk of not detecting a positive batch is low.

### Notes:

- Broilers or fish receive water treatment (group treatment), so testing 3-5 animals per batch gives a good estimate of the antibiotic level in the flock.
- Beef cattle is treated mainly individually, so here the test frequency should be higher (5-10%).
- Pigs can be treated individually or as a group. When 5 pigs per batch of 100 are tested, at least 3 should test negative with the Premi®Test.

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## How to stock the samples?

### For analyses within 48 h:

Store sample in refrigerator (4°C).

### For storage longer than 48 h

Freeze at - 18°C or below, preferably - 32°C or below.

### Notes:

- Do not stock the samples at room temperature, as this will increase bacterial growth in the sample and/or formation of by-products. This may cause false positive results (due to formation of inhibiting compounds) or false negative results (due to enzyme production by bacteria e.g. beta-lactamase).
- Avoid multiple freeze-thaw cycles; this may lead to a decrease in antibiotic stability.

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## How to prepare frozen samples?

### Option 1

Thaw the samples at room temperature

### Option 2

Thaw the sample in a water bath of 65 °C.

### Notes:

- Do not use the liquid at the bottom of the flask or plastic bag!
- Watch out for water crystals in the frozen sample! Water can give false positive results!
- Do not thaw the samples in the refrigerator for too long!
- If you use a microwave oven, preferably heat the sample at 160 Watt for 90 seconds.

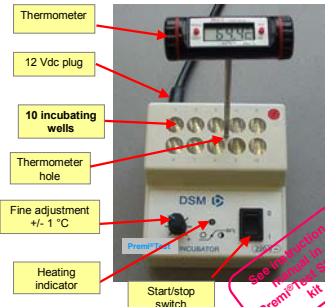
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## How to use the incubator ?

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### Notes:

- Plug in the Premi<sup>®</sup>Test incubator (220 or 110 Volt).
- It will take at least 5 minutes to reach a temperature of 64°C.
- Use the incubator in a room with a constant temperature (+10 to +35°C) and do not place it near an open window or in a draught.
- For best results, it is recommended NOT to insert new cold vials during a test run as this may lower the temperature.
- The Premi<sup>®</sup>Test incubator has CE and NF-EN certifications.
- Premi<sup>®</sup>Test Incubator should be stored and transported dry at a constant temperature between 4 and 35°C. Avoid freezing and strong mechanical shocks.

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## How to prepare poultry and fish samples to get enough juice?

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Sometimes it is very difficult to get enough juice!

In these cases it is recommended to freeze the samples and then thaw them in a water bath at 65°C.

Use the Multipress.

### Notes:

- It is very difficult to squeeze juice from meat from a freshly slaughtered animal.
- Heat the meat press either under warm tap-water or in the oven before squeezing.
- During squeezing turn the meat press upside down.

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## How to prepare the negative control?

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For a negative control, use meat from animals that were not given antibiotics.

Test this "untreated meat" first: if the Premi<sup>®</sup>Test result is negative, you can press more juice from this meat and use it for the negative control.

Store small quantities of meat juice (bags or cryotubes) in a freezer at -18 °C.

These negative controls can be stored for 6 months.

The shelf life increases to 12 months when these samples are stored at -40 °C.

It is advised to use a negative control of the species to be tested in each analysis: when the negative changes color, the samples should be red.

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### Notes:

- **DO NOT USE WATER as negative control!**
- Unlike meat juice, water contains no nutrients, which means the spores of the Bacillus will develop less readily!

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## How to prepare the positive control?

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### Start with "negative" meat juice

Example: Penicillin

Formula: C<sub>16</sub>H<sub>17</sub>KN<sub>2</sub>O<sub>6</sub>S MW: 372.5 g/mol  
Purity: 100% Correction for K (39.1 g/mol): 10.5%  
Corrected Purity: 89.5%  
Correction factor: 1 / 0.895 = 1.117

Preparation of Stock (1.000 ppb = 1 µg/ml)

- Weight: 10 mg \* (correction factor for purity) = 10 mg \* 1.117 = 11.17 mg

- Weigh out 11.17 mg of Penicillin and make up to 100 ml with distilled water

- Take 1 ml of this solution (100 µg/ml) and make up to 100 ml with distilled water (1 µg/ml)

Spiking meat or fish juice with stock solution

- Take 5 ml of meat or fish juice and add 50 µl of the stock solution.

- The positive control contains 10 ppb penicillin in the stock solution.

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### Notes:

- Divide freshly prepared positive standards into small portions (1-5 ml), e.g. in cryotubes.
- The positive control can be stored at -30 °C for 6 months.
- Do not freeze and thaw a standard for a second time, since this will inactivate the antibiotic activity.
- Use the positive standard once every month.

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## How to prepare eggs?

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The protocol for eggs requires a pre-incubation at 80°C for 10 minutes.

This is possible with the Premi<sup>®</sup>Test 2-Step Incubator.



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### Notes:

- The detection limits for eggs differ slightly from those for meat and fish (see the detection limits per matrix).
- Use the mixture of the egg-yolk and the egg-white!

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## How to use the Premi<sup>®</sup>Test for feed?

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Grind the feed in a mortar.

Take 10 grams of the ground feed.

Add 30 ml of water.

Stir for half an hour at room temperature.

Transfer 100 µl of the suspension onto the agar in the vial.

Pre-incubate at 80°C for 10 minutes in the 2-Step Premi<sup>®</sup>Test Incubator.

After this heat pre-treatment, incubate the Premi<sup>®</sup>Test at the required temperature of 64°C.

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### Notes:

- The detection limits for feed are totally different from those for meat, due to the dilution step!
- Cut a small part from the pipet tip to avoid blocking.
- There is no washing step in this protocol: the feed forms a gel during the 80-degree pre-incubation.

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## We wish you success in using the Premi®Test!

If you have any other queries or technical questions please contact us by e-mail:

[info.premitest@dsm.com](mailto:info.premitest@dsm.com)

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## Sensitivity of the Premi®Test in meat (µg/l)

Group	Substance	Chicken	Pork	Beef
<b>β-Lactams</b>	Amoxicillin	5	5	5
	Ampicillin	5	5	5
	Penicillin	2.5	2.5	2.5
<b>Cephalosporins</b>	Cefquinome	75	100	100
	Ceftiofur	400-600	400-600	400-600
<b>Macrolides</b>	Tylosin *	50-100	50-100	50-100
	Erythromycin	100	100	100
	Lincomycin	100	100	100
	Spiramycin	1000	1000	1000
<b>Tetracyclines</b>	Chlortetracycline	100	100	100
	Oxytetracycline *	200-400	200-400	200-400
	Doxycycline	100	100	100
<b>Sulphonamides</b>	Sulphadiazine *	200-400	200-400	200-400
	Sulphamethazine	75	75	100
<b>Aminoglycosides</b>	Gentamycin	100	100	100
	Streptomycin	1500	1500	3000
<b>Quinolones</b>	Enrofloxacin	>800	>800	>800
	Flumequine	>100	>100	>100
<b>Polypeptides</b>	Virginiamycin	500	500	500
	Bactrac	500	500	500

\* Results of external validation studies conducted at TNO (Zeevoed) and internal validation study of DSM Food Recalculates, Delft, The Netherlands (TNO - Dutch Organisation for Applied Scientific Research)  
\* Detection limit mentioned in the MRL/NoC certificate

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## Sensitivity of the Premi®Test in fish (µg/l)

Group	Substance	Eel	Salmon	Trout
<b>β-Lactams</b>	Amoxicillin	5	5	5
	Ampicillin	5	5	5
	Penicillin	5	5	5
<b>Cephalosporins</b>	Cefquinome	200	200	200
	Ceftiofur	200	200	200
<b>Macrolides</b>	Tylosin	75	100	75
	Erythromycin	200	200	200
	Lincomycin	200	300	200
	Spiramycin	750	1000	750
<b>Tetracyclines</b>	Chlortetracycline	200	200	200
	Oxytetracycline	200	200	200
	Doxycycline	150	150	150
<b>Sulphonamides</b>	Sulphamethazine	75	75	75
	Sulphadiazine	75	75	75
<b>Aminoglycosides</b>	Gentamycin	200	200	300
	Streptomycin	1500	3000	3000
	Neomycin	300	300	300
<b>Quinolones</b>	Enrofloxacin	>4000	>4000	4000
	Flumequine	1500	1500	1200
<b>Other</b>	Florfenicol	200	450	450
	Chloramphenicol	2500	2500	3000
	Fluoroquinolones	12,500.5	12,500.5	12,500.5
	Chloramphenicol	>1500	1500	1500

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## Sensitivity of the Premi®Test in eggs (µg/l)

Group	Substance	Egg
<b>β-Lactams</b>	Amoxicillin	5
	Ampicillin	5
	Penicillin	2.5
	Oxacillin	100
<b>Cephalosporins</b>	Ceftiofur	400
<b>Macrolides</b>	Tylosin	100
	Erythromycin	50
<b>Tetracyclines</b>	Chlortetracycline	600
	Tetracycline	200
	Oxytetracycline	400
	Doxycycline	200
<b>Sulphonamides</b>	Sulphamethazine	25
	Sulphadiazine	25
	Sulfamethoxazole	25
<b>Aminoglycosides</b>	Gentamycin	100
	Streptomycin	1000
	Neomycin	300
<b>Other</b>	Chloramphenicol	2500

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## Sensitivity Premi®Test compared to the MRL (µg/l)

Group	Substance	PremiTest detection limits	Maximum residue Level (in the EU for muscles tissues)
<b>β-Lactams</b>	Amoxicillin	5	50
	Ampicillin	5	50
	β-Penicillin	2.5	50
<b>Cephalosporins</b>	Cefquinome	75	50
	Ceftiofur	100	1000
<b>Macrolides</b>	Tylosine	50	100
	Erythromycin	100	200
	Lincomycin	100	100
	Spiramycin	1000	200 <sup>(1)</sup> 250 <sup>(2)</sup>
<b>Tetracyclines</b>	Chlortetracycline	100	100
	Oxytetracycline	100	100
	Doxycycline	100	100
<b>Sulphonamides</b>	Sulphamethazine	75	100
	Sulphadiazine	75	100
<b>Aminoglycosides</b>	Gentamycin	100	50 <sup>(1,2)</sup>
	Streptomycin	1500	500
	Neomycin	300	500
<b>Quinolones</b>	Enrofloxacin	>600	100
	Flumequine	>100	200 <sup>(1,2)</sup> 400 <sup>(3)</sup>
<b>Polypeptides</b>	Virginiamycin	500	0
	Bactrac	500	0
	Chloramphenicol	2500	0
<b>Other</b>	Florfenicol	<100	200 <sup>(1)</sup> 300 <sup>(2)</sup> 100 <sup>(3)</sup>

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1) beef, 2) pork,  
3) poultry