

Premi®Test

Art. No. R3900/R3925





Trouble shooting guide 

Premi®Test

Welcome to the Premi®Test troubleshooting guide! This guide will help you identify and resolve common issues encountered during this analysis, ensuring accurate and reliable results. Covering sample preparation, assay reagents, testing environment and equipment, each section presents observed issues, potential root causes and recommended actions for resolution.

Our team is here to support you! If you need any additional assistance or have further questions while troubleshooting, please do not hesitate to contact us. We are dedicated to helping you achieve successful outcomes in your analysis.

Test procedure

Procedure	Notes and advices
<p>Step 1: Prepare the ampoules</p> 	<ul style="list-style-type: none"> • Remove the required amount of ampoules from the frame. • Be careful not to damage the foil of the remaining ampoules. • Use one additional ampoule for the negative control, which is a sample containing no antibiotics.
<p>Step 2: Obtain meat juice</p> <p>Take approx. 2 cm³ of lean meat. Extract about 250 µL of meat juice using the meat press delivered with the Premi®Test Starter Kit (Art. No. ZPT-2000).</p> 	<p>Heat the meat press in an oven or under warm tap water before usage. Increase the pressure slowly and hold constant. Repeat this step with new pieces of meat until approx. 200 µL of meat juice is obtained.</p> <ul style="list-style-type: none"> • It might be necessary, to turn the meat press upside down after some time. • This technique works best with fresh red meat! • 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. • Do not use minced or ground meat! • Rinse the meat press sufficiently with distilled water for cleaning.

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Step 3: Open the ampoule

Label the ampoules and open the ampoules with one corner of the green frame.



It might be necessary to remove all of the pinched foil of the opened ampoule to allow proper rinsing.

Step 4: Transfer the meat juice in the ampoule

Pipette 100 μ L of juice slowly onto the agar in the ampoule.



- Dip the pipette into the liquid and decrease the pressure on the bubble to allow the stem to fill with the meat juice.
- Add the meat juice to the pipette by squeezing the smaller upper bulb.
- By fully squeezing the top bulb, approx. 100 μ L of the meat juice will be released.
- (Note: A small excess of liquid may remain in the lower bulb.)
- Of course you can use a calibrated pipette from your lab.
- Take care not to touch the agar!
- Use a new pipette for each sample!
- Make sure that the pipette tip is not blocked by small pieces of meat in the juice!
- 100 μ L is needed to get the right sensitivity.

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Step 5: Prediffusion

Allow to stand at room temperature (20 - 25 °C) for 20 min for prediffusion.



- Do not allow the samples to stand at temperatures outside this range.
- Plug in the Premi®Test Incubator, so it can preheat to 64 °C.

Step 6: Get the juice out of the ampoule

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



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

Step 7: Close the ampoule

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



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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Step 8: Start the incubation

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



- Did you remember to plug in the incubator at the prediffusion stage (see step 5)?
- Alternatively, you can incubate the ampoules in a water bath with a floating rack.

Step 9: Remove the ampoule

Withdraw the ampoules from the incubator after approx. 2.5 h (as explained in the manual). Determine the color of the lower 2/3 part of the solid agar.



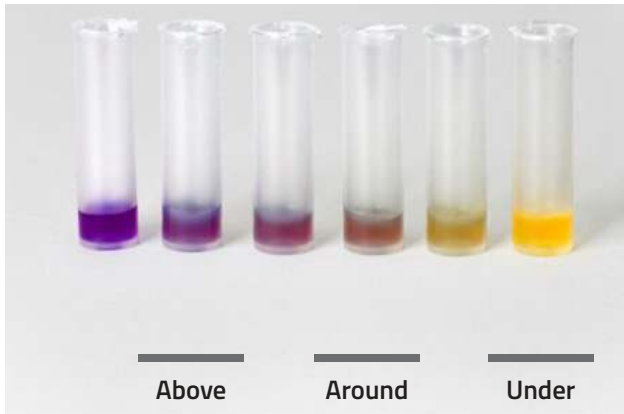
- At the moment the negative control of one species changes into yellow, read all the samples of the same species!
- If all samples are still purple or suspected, leave them in the incubator for another 5 min.
- After these 5 min, read again the color of the bottom part of the contents of the ampoules.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



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Step 10: Determine the color visually

Detection limit of the Premi®Test



- **Above:**
No color change = no growth of *Bacillus* = presence of an antibiotic
- **Under:**
Change of color = growth of *Bacillus* = absence of an antibiotic

- Determine the color of the lower 2/3 of the contents of the ampoule.
- A purple border can appear at the top of the contents.
- The top of the contents can also be colored by the meat juice.
- Be careful: color reading errors can lead to major problems in the results.

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Frequently Asked Questions

FAQ	Note
<p>What samples to take?</p> <p>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.</p> <p>Examples of suitable samples:</p> <ul style="list-style-type: none"> • pork: diaphragm • beef: lean meat • poultry: filet 	<p>Do not use:</p> <ul style="list-style-type: none"> • the tongue • the kidney, except when kidney is to be tested (in that case use the recommended protocol for kidney samples) • the liver, except when liver is to be tested (in that case use the recommended protocol for liver samples)
<p>How much sample?</p> <p>The minimum volume of a sample is 2 cm³. 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.</p>	<ul style="list-style-type: none"> • Never refreeze defrosted samples! • Prevent surface contact between different samples.
<p>How many samples per batch?</p> <p>If the animals were given antibiotics collectively at the farm (e.g. through the feed in the case of broilers or pigs), 2 - 5 samples / animal per batch can be taken. The heterogeneity within a group of animals is predictable, but animal to animal variation is not. Using 5 samples per batch, the risk of not detecting a positive batch is low</p>	<ul style="list-style-type: none"> • 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 tests should be negative with the Premi®Test.
<p>How to stock the samples?</p> <p>For analyses within 48 h: Store sample in refrigerator (4 °C).</p> <p>For storage longer than 48 h: Freeze at least at -18 °C. Preferably below -32 °C.</p>	<ul style="list-style-type: none"> • Do not stock the samples at room temperature (20 - 25 °C), 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 (20 - 25 °C).

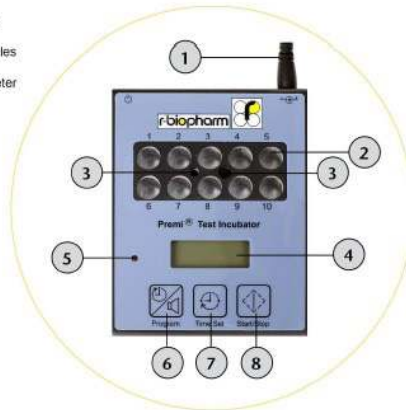
Option 2:

Thaw the samples in a water bath at 65 °C.

- 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 sec.

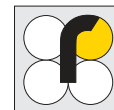
How to use the incubator?

- 1 Connection to power supply
- 2 Holes for Premi®Test ampoules
- 3 Holes for external thermometer
- 4 Display
- 5 Status LED
- 6 Program-Button
- 7 Time Set-Button
- 8 Start/Stop-Button



- Please refer to the manual for more information.
- Plug in the Premi®Test incubator (220 or 110 Volt).
- It will take at least 5 min to reach a temperature of 64 °C.
- Use the incubator in a room with a constant temperature (10 - 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.
- Premi®Test Incubator should be stored and transported dry at a constant temperature between 5 - 35 °C. Avoid freezing and strong mechanical shocks.





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How to prepare the negative control?	
<div data-bbox="226 607 571 1106" style="border: 1px solid black; padding: 5px;"> <p>Recommendation</p> <p>Premi®Test (Art. No. R3900/R3925) Preliminary supplement Preparation of negative control samples</p> <p>Please see the instruction for use (Premi®Test, Art. No. R3900/R3925).</p> <p>Notifications</p> <p>For the use of Premi®Test, the negative control samples are obligatory as indication for the correct incubation time to determine the results. Use only samples which verifiably contain no antibiotic residues. Use only fresh samples for the preparation of negative control samples. Wash hands and clean working surface before starting the procedure.</p> <p>Reagents and equipment required (for this method, not mentioned in the instruction for use) As described in the related instruction for use.</p> <p>Procedure</p> <ul style="list-style-type: none"> Follow the instructions for the preparation of samples in accordance to the respective application note. Adjust the samples in portions in a multiplicative volume of 100 µL (e.g. 3 x 100 µL = 300 µL). Make a test run with one of these samples in accordance to the respective application note. <p>Storage</p> <ul style="list-style-type: none"> Store the samples in a freezer at -20 °C (-4 °F) for a maximum of 6 months. The shelf life can be increased to 12 months when samples are stored at -40 °C (-40 °F). Do not freeze and thaw the negative control for a second time. <p><small>Disclaimer: This recommendation given is based on general experience, which has neither been verified nor validated by us. The recipient of this recommendation shall verify and obtain any recommendations given by third parties. We do not claim to guarantee any specific results. Respective liability, including any and all accessories and liabilities of any kind (including without limitation warranties on non-interruption of technical property rights of any third party) with respect to any and all information given in this recommendation.</small></p> <p><small>© Biopharm - An der Innon-Bergstraße 11, 42699 Solingen, Germany • Email: sales@biopharm.de • food@biopharm.com</small></p> </div>	<ul style="list-style-type: none"> For a negative control, use meat from animals that were not treated with antibiotics. Test this meat first: if the Premi®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 read. Do not use water as negative control! Unlike meat juice, water contains no nutrients, which means the spores of the <i>Bacillus</i> will develop less readily.
How to prepare the positive control?	
<p>Instructions for the preparation of positive controls are available for:</p> <ul style="list-style-type: none"> macrolides β-lactam antibiotics sulfonamides tetracyclines 	<ul style="list-style-type: none"> 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.