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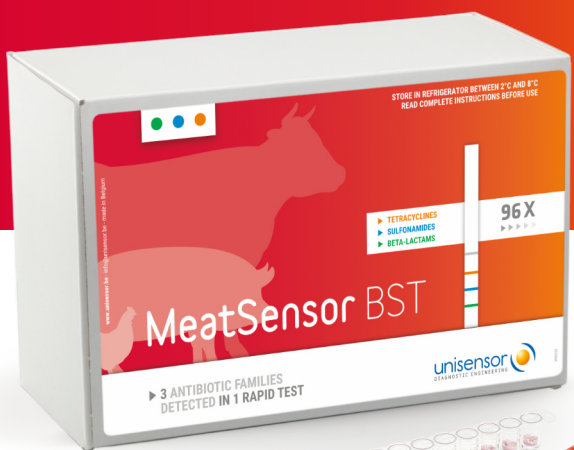
DIAGNOSTIC ENGINEERING

MeatSensor BST

OPERATING INSTRUCTIONS



RAPID TEST STRIPS DETECTING BETA-LACTAMS, SULFONAMIDES & TETRACYCLINES IN MEAT

PRODUCT REFERENCE - KIT108

ENGLISH

Version 1.1 - March 05, 2021
Designed and produced in Belgium

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ENGLISH

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CHAPTER 1 - INTRODUCTION

MeatSensor BST is a rapid qualitative test that allows you to simultaneously detect and discriminate the presence of ß-lactams, Sulfonamides and Tetracyclines molecules in a meat sample (muscle).

SUMMARY OF THE PROTOCOL

1. **Prepare the meat (muscle) sample extract using the provided extraction buffer (see complete procedure at Page 7).**
2. **Add 200 µl of meat (muscle) extract into one reagent microwell placed on the incubator and mix to homogeneity.**
3. **Incubate 3 minutes at 40°C.**
4. **Dip one dipstick into each microwell.**
5. **Continue incubating for 4 minutes at 40°C.**
6. **Take the dipstick out of the incubator and stop the reaction by removing the sample pad.**
7. **Interpret the results immediately after the end of the test or read the result using the ReadSensor 2 (within a 2-minutes time frame).**

REACTION MECHANISM

MeatSensor BST is a competitive test involving generic receptors and monoclonal antibodies in one single operation. The test requires the use of two components:

- The first component is a microwell containing predetermined amounts of both receptors and antibodies linked to gold particles.
- The second is a dipstick made up of a set of membranes with specific capture lines (three test lines and one control line).

For a valid test, the upper red control line has to be visible after the second incubation (see Chapter 5).

The other three are specific test lines placed below the control line. The line for ß-lactam antibiotics (penicillins and cephalosporins) is located below the sulfonamides line while the line related to tetracyclines is located above it.

When the reagent from the microwell is reconstituted with a meat extract, both receptors and monoclonal antibodies will bind the corresponding analytes, if present, during the first 3-minutes incubation at 40°C. Afterwards, when the dipstick is dipped into the reconstituted microwell, the liquid starts running vertically on the dipstick and passes through the capture zones.

When the sample is free of antibiotics, a color development occurs at the specific test lines, indicating the absence of the targeted analytes in the meat extract. On the contrary, the presence of antibiotic(s) in the extract will impair the colored signal to appear at the specific test lines.

CHAPTER 2 - COMPOSITION OF THE KIT

MeatSensor BST Kits contain everything needed to perform 96 measurements.

TEST TUBES

12 tubes each with 1 strip of 8 reagent microwells and 8 dipsticks

PIPETTE

1 micropipette of 200 µl and disposable tips

CERTIFICATE

1 Certificate of Compliance

EXTRACTION BUFFER

1 x 300 ml - REF. PIN463 (KIT110 containing 1 x 300 ml bottle can also be acquired separately)

ADDITIONAL MATERIAL NEEDED (NOT INCLUDED)

- HeatSensor (40°C incubation, refer to the HeatSensor user manual)
- ReadSensor 2 (optional, refer to the ReadSensor 2 user manual)
- Material for sample preparation (weight scale, mixer, tubes, pipettes, vortex, centrifuge)
- Timer/Watch
- MeatSensor BST Certificate of Analysis and Operating Procedure can be downloaded in the Download Center on Unisensor website (www.unisensor.be)

CHAPTER 3 - GENERAL REMARKS

- Store the kit at a temperature between 2°C and 8°C in a controlled fridge.
- Avoid repeated temperature variation.
- Avoid exposure of the reagents to moisture and light.
- Do not mix components of kits from different batches.
- Do not use the kit after the expiration date.
- Avoid touching the membrane on the dipstick.
- Avoid leak of reagent during sample addition and mixing step.
- Empty one tube before opening another tube.
- Close accurately the tube after use.
- Use lean meat without fat for the extraction.
- The temperature to perform the test is 40°C ± 1°C. Use the “HeatSensor”. Any other type of incubator is not appropriate to perform the MeatSensor BST assay. The use of other incubators than that indicated in this document is the responsibility of the customer and does not bind Unisensor (see HeatSensor User manual).
- Avoid direct air flow on the dipstick, this impacts the migration rate and might causes wrong results.
- When drying, the color intensities of the lines will become sharper. Interpret the results immediately after the end of the test or read the result using the ReadSensor 2 (within a 2-minutes time frame).
- When a positive result is recorded, the test result should be confirmed by performing two more tests.

CHAPTER 4 - TESTING PROCEDURE

PREPARATION

1. Instructions and site preparation

- Read the instruction carefully.
- Choose a clean and dry place to perform the test.
- Wash and dry your hands.
- The reader and the incubator must be clean.

2. Start the instruments

Switch on the « HeatSensor » (DUO or OCTO) and select the program set at the right temperature (40°C) and timing (3 min + 4 min) (see HeatSensor user manual). Wait until the temperature has stabilized at 40°C.

Switch on the « ReadSensor 2» if instrumental reading is chosen for result interpretation (see ReadSensor user manual) and make sure that the check / calibration was performed according to the user manual.

3. Take the kit out of the fridge

Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the room temperature.

4. Preparation of samples

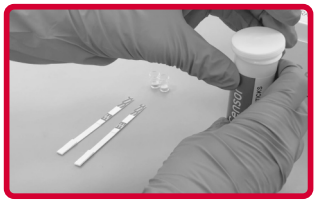
- Identify all samples and tubing with a specific number or code
- Grind the lean meat until obtaining a minced meat aspect (not mashed).
- Weigh 2 g of minced meat in a centrifugal tube.
- Add 3 ml of Extraction Buffer and mix vigorously for 1 minute.
- Transfer 1 ml into a 1.5 ml tube and centrifuge at full speed for 3 min at room temperature to remove muscle debris.

NOTE

- Extraction and testing have to be performed sequentially.
- Do not store the extracts for later analysis.

CHAPTER 4 - TESTING PROCEDURE

TESTING STEPS



5. Open one plastic tube

Open a tube and take out as many microwells and dipsticks as there are meat (muscle) samples to be tested. Be careful to leave the protecting film on the unused microwells. Immediately put the remaining reagents back into the plastic tube without damaging the dipsticks, close the tube and make sure it is tightly sealed. **If you are not planning to perform any other test within the day, put everything back into the kit and store it in a fridge at a temperature ranging from 2°C to 8°C.**

Testing more than 8 samples at a time is not recommended. In the case many samples have to be analyzed, try to perform the tests in cascade to avoid any delays occurring during the manipulations. For more than 3 samples, consider using a multichannel pipette. Make sure you have the same incubation time for each sample.

6. Microwell(s) on incubator

Place the microwell(s) in the heating block which shows 40°C.

7. Transfer 200 µl of meat extract

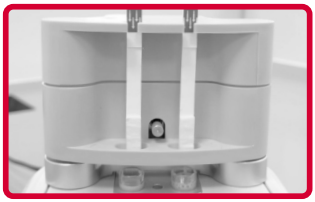
For each sample, place a new disposable tip on the micropipette and transfer 200 µl of meat (muscle) extract into each of the microwells. Then mix immediately by pipetting up and down 10 times.

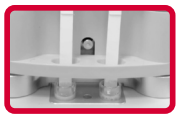
WARNING: when reagents and the extract are in contact, the reaction begins. Make sure the time for sample preparation and handling is minimal.

Then, immediately push the start (run) button on the Heatsensor. The initial 3-minutes incubation starts.

CHAPTER 4 - TESTING PROCEDURE

TESTING STEPS





8. Place dipsticks into the holder

Place the dipsticks into the HeatSensor holder (DUO/OCTO).

After the initial incubation, the dipsticks will be dipped automatically into the microwell.

The incubation will proceed for the next 4 minutes.

9. Sample pad removal

When the migration is over, take the dipsticks out from the microwells and holder.

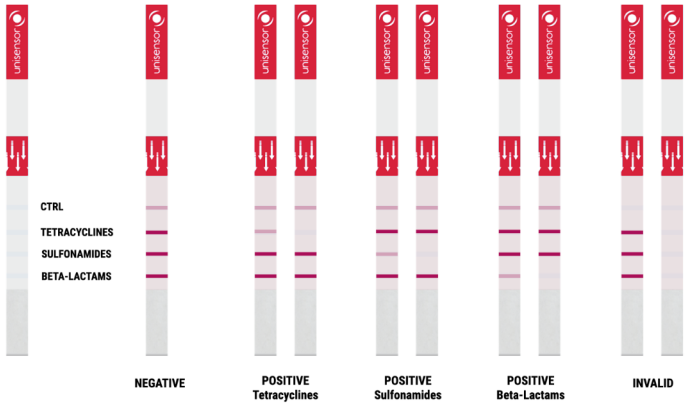
Gently remove the sample pad, without damaging the central membrane, to stop the reaction.

10. Interpretation of the results

Proceed to the interpretation with the ReadSensor 2 (see CHAPTER 5) within maximum 2 minutes.

CHAPTER 5 - RESULTS

VISUAL INTERPRETATION



1. Check whether the top control line is present. If it is not, consider the analysis as invalid and do not continue any interpretation.
2. When the top control line can be seen, interpret the three test lines as follows: examine one test line at a time and compare the color intensity of the test line with the color intensity of the control line.
3. Start with the bottom line of Beta-lactam antibiotics for example:
   - If the test line color intensity is darker than the control line, the result is **NEGATIVE**, which means that, given the sensitivity of the test, the meat sample contains no antibiotics or antibiotics at a lower level than the value stated in the enclosed **TABLE A**.
   - If the test line color intensity is similar, lighter (or absent) than the control line, the result is **POSITIVE**, which means that, given the sensitivity of the test, the meat sample contains antibiotics at or above the detection values stated in the enclosed **TABLE A**.
4. When you have interpreted one test line, do the same for the other lines.
5. Write down your assessment on each of the dipsticks.

NOTE

- **IF YOU HESITATE, CONSIDER THE SAMPLE AS POSITIVE AND CONFIRM YOUR INTERPRETATION BY PERFORMING TWO MORE TESTS.**
- **DIPSTICKS CAN BE ARCHIVED AS A PERMANENT RECORD IF THE SAMPLE PAD HAS BEEN REMOVED JUST AFTER THE END OF THE TEST. ALLOW THE STRIP TO DRY BEFORE STORAGE. PLEASE NOTE THAT THE COLOR INTENSITY CAN VARY WITH DRYING.**

CHAPTER 5 - RESULTS

USING READSENSOR 2

1. Reading Method

Make sure you have entered the appropriate and latest version available of the reading Method into your ReadSensor 2.

The reading Method files are available in Unisensor website's Download Center at:
https://unisensor.be/en/fileaccess/download

2. Reading & Interpretation

- If the control is not visible (with or without the presence of test lines), do consider the test as not valid, don't interpret the result and directly run another test. The ReadSensor will confirm it by giving an INVALID result.
- You should read the dipstick within 2 minutes after performing the test.
- On the basis of a ratio obtained between the test and control line color's intensities, the reader will interpret the result as follows:

RESULTS INTERPRETATION		
TEST RESULT	NEGATIVE (NEG)	POSITIVE (POS)
INTERPRETATION	CONTAMINATION ABSENT OR BELOW THE LIMIT OF DETECTION	CONTAMINATION AT OR ABOVE THE LIMIT OF DETECTION

CHAPTER 5 - RESULTS

TABLE A: PRELIMINARY ESTIMATION OF LIMITS OF DETECTION*

B-LACTAMS		
PENICILLINS		
ANTIBIOTIC	**MRL (PPB)**	**LOD (PPB)**
Ampicillin	50	20 - 30
Penicillin G	50	15 - 25
Amoxicillin	50	20 - 30
Oxacillin	300	60 - 75
Cloxacillin	300	60 - 75
Dicloxacillin	300	35 - 50
Nafcillin	-	350 - 450
CEPHALOSPORINS		
ANTIBIOTIC	**MRL (PPB)**	**LOD (PPB)**
Ceftiofur	Sum 1000	100 - 125
Desfuroyl Ceftiofur		≤ 1000
Cefquinome	50	50
Cefazolin	-	75 - 100
Cefalonium	-	50 - 75

TETRACYCLINES		
ANTIBIOTIC	**MRL (PPB)**	**LOD (PPB)**
Tetracycline	100	60 - 80
Oxytetracycline	100	80 - 100
Chlortetracycline	100	25
Doxycycline	100	20 - 25
SULFONAMIDES		
ANTIBIOTIC	**MRL (PPB)**	**LOD (PPB)**
Sulfadiazine	Sum 100	≤ 100
Sulfadimethoxine		≤ 100
Sulfamethazine		≤ 5

* LOD PENDING VALIDATION - MRL values refer to porcine muscle (µg/kg) according to COMMISSION REGULATION (EU) No 37/2010

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