



6a. Specification sheet

DSM Food Specialties B.V.

P.O. Box 1
2600 MA Delft
The Netherlands

Delvotest® T

www.dsm.com

Description Delvotest® T is a standard diffusion test for the detection of residues of antibacterial substances (antibiotics and sulphonamides) in milk. The test consists of ampoules or plates of 96 wells each containing a solid agar medium seeded a standardised number of spores of *Bacillus stearothermophilus* var. *calidolactis* together with required nutrients for growth purposes and an antifolate trimethoprim. The medium is coloured purple by the pH indicator bromocresol purple. Milk samples which are free from antibacterial substances, or contain them below specified levels will, when added to the test at the level of 0.1 ml and incubated at 64°C allow germination and growth of the bacteria. This will lead to a change in colour of the indicator from purple to yellow. When the milk sample contains antibacterial substances at or above the test sensitivity, growth is inhibited and as a result the colour remains predominantly purple.

Content The Delvotest T is available in the following packaging format
- Delvotest® T 100 Ampoules

Sensitivity The minimum test duration is defined as the incubation time required for the colour of the test to change from purple to yellow with inhibitor free milk.
The test duration is 3 hours +/- 15 minutes at 64°C +/- 2°C at the time of product release.

The sensitivity at the minimum test duration with inhibitor free milk is:

	Penicillin G		Sulfadiazine	Oxytetracycline
Concentration	1ppb	4ppb	100 ppb	100 ppb
Observed colour	Yellow to yellow/purple	Purple	Predominantly purple	Predominantly purple

Infection control No microbial growth for 1 week at 30°C.

Storage When stored upright in the original packaging, in the dark and at a constant temperature below 8°C prevented from freezing (preferably between 4-8°C) the shelf life of the Delvotest® T is 12 months from the date of manufacture. This date is indicated on the label.



6b. Instruction for use

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Delvotest® T Ampoules



1. Remove the required amount of ampoules from the frame. Be careful not to damage the foil of remaining ampoules.



2. Open ampoules by punching a hole in the aluminum foil with the corner of the ampoule frame or a pair of scissors. Mark the ampoules with a number for sample identification.



3. Take a fresh disposable pipette for each milk sample. Do not re-use pipettes. Do not touch the tip-end, which will be in contact with the milk.



4. Add the milk into the pipette by squeezing the smaller upper bulb once, hold it, dip the pipette tip about 1 cm into the milk sample. Then release pressure on the bulb and the pipette (stem) will fill itself with the appropriate volume (0.1 ml) of milk.

Note: After pipetting, the little surplus of milk is caught into the pipette reservoir (the small lower bulb). Repeat the pipetting when there is no milk in the reservoir.



5. Transfer the milk samples by gently and totally squeezing the same upper bulb, adding the milk straight onto the agar medium. **Note:** The little surplus of milk in the lower bulb will remain.

6. Check the temperature of the incubator (64°C +/- 2°C). Put the ampoules into the incubator. Use a control sample or record the time and set timer.

7. Read the colour of the solid agar in the ampoules after the required incubation time. Fixed reading time is 3 hours and 15 minutes or use control time reading.

Points of Attention:

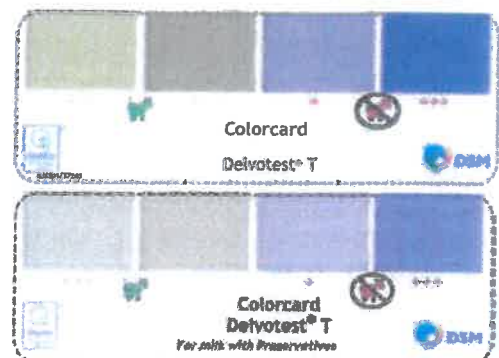
1- Use the enclosed color card to read the test results.

2- When using control time, the negative control sample is not what one would qualify as "bright yellow". Refer to the color card for the correct yellow color indicating negative (a specific color card is available when analyzing samples preserved with an additive such as Azidiol). A too long incubation time reduces the sensitivity of the test.

The incubation time for goat & ewe milk can take about 10-30 minutes more than for cow milk.

3- To allow more time for reading after incubation, the tests can be dipped it in a cold bath of water with ice. The cold stops further color change.

4- The 1/3 upper part of the agar gel in the ampoule might remain purple while the 2/3 lower part of the gel is yellow. The test result is then negative.





Delvotest Manual V1



6c. Instruction for use

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Delvotest® T Plates



1. Determine the number of plates and/or blocks of 16 wells needed and cut them off with a sharp knife or scissors. Be careful not to damage remaining blocks.

2. Remove the aluminium foil completely.

3. Pipette the negative control and the sample to be tested into the wells. Identify the position of each sample by the letters and figures on the edge of multi plates.



4. Seal the blocks with the adhesive strips or sheets supplied with the test kit.

5. Float the blocks in a stirred, lidded water bath or plate incubator - preheated to 64°C ±2°C. Set timer for (2Hours, 45 minutes and check for control time (2h45-3H15)

6. When the negative control has turned yellow withdraw the blocks/plates from the water bath and read the results from the bottom of the blocks



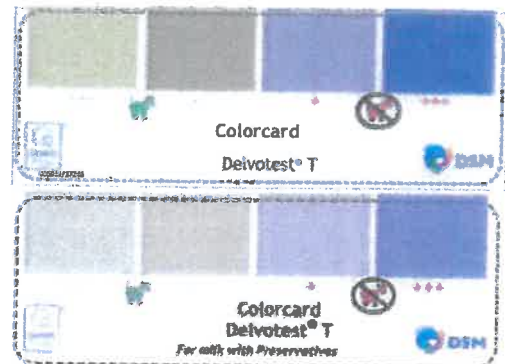
Points of Attention:

- 1- Use the enclosed color card to read the test results.
- 2- The block freshly incubated can be automatically analyzed by the system DelvoScan: Simply remove the plates from the water bath, dry the plates using a towel and place them at the required positions on the scanner. Run the DelvoScan software.
- 3- The control time is the time for the test to become yellow with antibiotic free milk. Refer to the color card for the correct yellow color indicating negative. A too long incubation time reduces the sensitivity of the test.

The incubation time for goat and ewe's milk takes about 10-30 min more than for cow milk.

4- To allow more time for reading after incubation, the tests can be dipped it in a cold bath of water with ice. The cold stops further color change.

5- The 1/3 upper part of the agar gel in the well might remain purple while the 2/3 lower part of the gel is yellow. The test result is then negative.





6d. Sensitivity

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Delvotest® T

Sensitivity of the Delvotest T

The table presented in this document is a list of sensitivity of the Delvotest® T for the most used antibiotics in the world. This list is not exhaustive. The test is sensitive to many more antibiotic drugs and sulfonamides.

The sensitivity corresponds to the concentration for which 95% of the samples analyzed are positive.

The best test sensitivity is reached when using the control time (3hours ± 5min). To measure at control time, use one test with negative control milk and stop the incubation of the test when the test with the control milk as turned yellow. Read then the result of the unknown samples.

The detection concentrations of the test when using a fix incubation time (3 hours) are very similar. There are no significant differences between plates and ampoules.

Antibiotic class	Antibiotic	MRL	CCB (ppb)	
			Ampoule	Break-Plate
Penicillins	Amoxicillin	4	4	4
	Ampicillin	4	4	3
	Penicillin G	4	2	2
	Cloxacillin	30	6	5
	Oxacillin	30	30	30
Tetracyclines	Oxytetracycline	100	100	80
	Chlortetracycline	100	150	152
	Tetracycline	100	70	75
	Doxycycline	(0)	50	40
Sulfonamides	Sulfamethazine	100	135	150
	Sulfathiazole	100	40	30
	Sulfadimethoxine	100	40	40
	Sulfadiazine	100	40	50
Macrolides	Tilmicosin	50	60	60
	Tylosin	50	35	35
	Erythromycin	40	160	150

Antibiotic class	Antibiotic	MRL	CCB (ppb)	
			Ampoule	Break-Plate
Aminoglycosidases	Neomycin	1500	60	110
	Gentamycin	100	65	80
	Kanamycin	150	1010	1310
	DH/Streptomycin	200	4240	5830
	Spectinomycin	200	2010	1850
Cephalosporins	Cephapirin	60	6	5
	Ceftiofur (pur)*	100	20	20
	Cefoperazone	50	40	40
	Cefalexin	100	30	20
	Cefquinome	20	40	40
Others	Lincomycin	150	220	180
	Chloramphenicol	(0)	4100	3080
	Trimethoprim	50	110	130
	Rifamixin	60	40	30
	Dapson	0	30	35

*Ceftiofur with metabolites has a detection limit about 4 times higher.