Ankar Milk Test 4 - KIT460

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Unisensor

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1. Foreword – Introduction

ANKAR Milk Test 4 is a rapid qualitative test designed to simultaneously detect and discriminate the presence or absence of **ß-lactams, tetracyclines, (dihydro)streptomycin, and chloramphenicol** residues in a raw cow milk sample.

Reaction Mechanism

ANKAR Milk Test 4 is a competitive immunoassay based on the interaction between specific receptors and generic monoclonal antibodies, performed in a single analytical procedure.

The test requires two components:

- The first component is a microwell containing predefined amounts of receptors and monoclonal antibodies conjugated to gold particles.
- The second component is a dipstick composed of a set of membranes with specific capture lines.

For a test to be considered valid, the upper red control line must be visible after the second incubation step.
Four additional lines located below the control line correspond to the specific test lines.

When the reagent in the microwell is reconstituted with a milk sample, both the receptors and the monoclonal antibodies bind to their corresponding analytes, if present, during the first incubation step (5 minutes at 40°C).

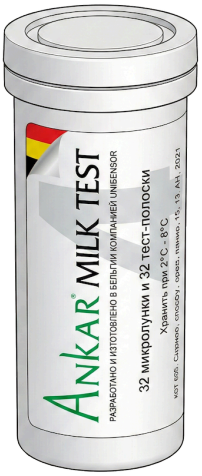
Subsequently, when the dipstick is immersed in the milk sample, the liquid migrates vertically along the membranes and passes through the capture zones.

In the absence of antibiotic residues in the sample, a colored signal develops at the specific capture lines, indicating a negative result. Conversely, when antibiotics are present in the sample, no colored signal appears at the corresponding test lines.

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2. Composition of the kit

ANKAR Milk Test 4 kit contains all reagents and materials required to perform 480/96 analyses. The kit is available in two packaging formats: **96 tests** and **480 tests**.

Component	Identification
15/3 plastic tubes, each containing 4 strips of 8 reagent microwells and 32 dipsticks	 
1 glass vial of **POSITIVE CONTROL** (red cap)	
1 glass vial of **NEGATIVE CONTROL** (green cap)	
Micropipette – 100 µL	
Disposable pipette tips (480±10/100±4 pcs.)	

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Certificate of Analysis	
Instructions for use	

ADDITIONAL EQUIPMENT REQUIRED TO PERFORM THE TEST

- HeatSensor incubator, Ankar TF, TIAS (incubation at 40°C; refer to the operating manual)
- ReadSensor 2 / ANKAR-100 reader (optional; refer to the reader operating manual)
- Distilled water
- Timer / watch

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3. Summary of protocol

1. Add 100 µl of milk into one reagent microwell and mix to homogeneity
2. Incubate 5 minutes at 40°C
3. Dip one dipstick into each microwell
4. Continue incubating for 5 minutes at 40°C
5. Remove the dipstick and stop the reaction by removing the sample pad
6. Read the results

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4. General remarks

- If an instrumental reading is chosen for result interpretation, the Readsensor 2 / Ankar 100 must be switched on before the analysis (see Readsensor 2 / Ankar 100 User Manual).
- At reception, store the bag in a dry place and at a low temperature between 2°C and 8°C. Before opening, let the plastic tubes reach room temperature and avoid exposure of the product to moisture and light.
- The milk sample must be liquid and homogeneous. There can be neither clots nor sedimentation phases. The ideal temperature of the milk sample, before the analysis, is between **4°C and 8°C**.
- Use the entire content of one tube before opening the next one.
- Do not mix components of kits from different batches.
- Avoid the loss of reagents during sample addition and mixing step.
- The best temperature of the incubator Heatsensor (or Ankar TF, TIAS) to perform the test is **40°C ± 1°C**. Any other type of incubator is not appropriate to perform the **ANKAR Milk Test 4 assay**. The use of other incubators than indicated in this document is the responsibility of the client and does not bind Unisensor (refer to the incubator User Manual for setting at right temperature and timing).
- The timing to perform the test is **5 minutes + 5 minutes**. No extra time is allowed.
- After the migration step, take out the dipsticks immediately, remove the sample pad and read the result immediately. Do not attempt to interpret the result after 5 minutes.
- When drying, the color intensities of the lines will become sharper.
- When a positive result is recorded, the test result must be confirmed by performing **two additional tests**.

Limitations of use

- The room temperature should be between 18°C and 24°C.
- Avoid to expose dipsticks to direct air flow like air conditioner, opened windows, etc. during migration.
- Respect recommended temperatures for milk samples storage (4°C to 8°C).
- Do not use other milks than fresh raw cow milk.
- Milk should be tested within 48 hours after milking.
- No transformed or powder milk can be tested.
- High fat samples might have an impact on the results.
- Level of somatic cells or bacterial presence might have an impact on results.
- To make **ANKAR Milk Test 4** measurements reliable, pH of the milk must be between 6.5 and 7.2.
- It is not recommended to use **ANKAR Milk Test 4** in environment where relative humidity is outside the range of 30%-80% (at 20°C).
- Do not leave dipsticks outside the tube for a long period before use.

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5. Testing procedure

- Before starting
- Testing steps
- Use of the control vials

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5.1. Before starting

This procedure is described to easily run one single sample or a set of many samples. In that case, try to perform the test in cascade and avoid any delays when mixing reagent and milk but also when adding and removing dipsticks.

Make sure you have the same incubation time and temperature for each sample. You shouldn't test more than **8 samples at a time** and, if there are more than 2 samples, you should use a multichannel pipette. With more than 8 samples we recommend to run series of maximum 8 samples.

Site preparation

Choose a clean and dry place to perform the test and wash and dry your hands before starting.

Start the incubator

Start the incubator (refer to the HeatSensor, Ankar TF, TIAS User Manual) and allow the temperature to stabilize at **40°C** before proceeding.



Take the kit out of the refrigerator

Before opening the reagents, remove the kit from the refrigerator and allow it to equilibrate to ambient temperature. During this time, carefully read the operating procedure. Determine the number of samples to be tested and assign an identification number to each milk sample tube.

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5.2. Testing steps

1. Open one plastic tube



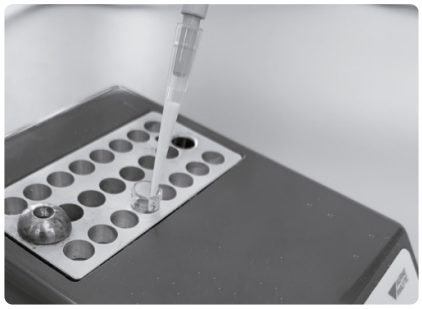
- Remove as many reagent microwells as there are milk samples to be tested (including positive and negative controls, if necessary).
- If you do not intend to use all the 8 microwells, leave the sealing plastic foil on the unused ones.
- Put back unused microwells immediately into the white tube without damaging the dipsticks. Close and make sure it is tightly sealed.
- The plastic tubes should always be well closed after reagents have been taken out.

2. Microwell(s) on incubator



- Place the microwell(s) into the heating block set at **40°C**.

3. Transfer 100 µL of milk



- Immediately transfer with a new tip 100 µl of milk into each of the microwells. Then mix by pipetting up and

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down 5 to 10 times.

> ! **WARNING:** When reagents and milk are in contact, the reaction begins.

- Then, immediately push the start (run) button on the HeatSensor, Ankar TF, TIAS incubator or use a timer. The 5-minutes countdown starts.

4. Place dipsticks into the microwells



- During the 5-minute incubation, open the same tube as before, remove the required number of dipsticks corresponding to the analyses in progress, and immediately close the tube.
- Place the dipsticks on a clean surface and identify them according to the milk sample IDs.
- At the end of the 5-minute incubation, manually insert each dipstick into the corresponding microwell.
- The **5-minute migration step** begins.

5. Sample pad removal



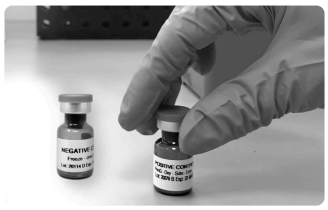
- At the end of the 5-minute migration, remove the dipsticks from the microwells.
- Immediately remove the sample pad and proceed with dipstick result interpretation.

> ✻ If no additional tests are planned for the same day, return all components to the kit and store it in a refrigerator at a temperature between **2°C and 8°C**.

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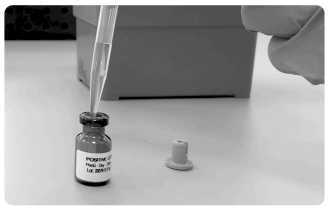
5.3. Use of the control vials

1. Take the negative and positive control vials



- Take one glass vial of **NEGATIVE CONTROL** (green cap) and one glass vial of **POSITIVE CONTROL** (red cap) from the kit.
- Remove the cap and rubber stopper from each glass vial.
- Avoid mixing or changing the respective stoppers of both positive and negative control vials.

2. Reconstitute with water



- Add **1 mL** of distilled or deionized water at ambient temperature to each vial.
- Replace the corresponding stopper on each control vial.
- Allow the solution to stand for **1 minute and a half** at ambient temperature.

3. Mix



- Shake each vial vigorously for **10 seconds**.
- Check that the sample is properly dissolved.
- Once reconstituted, the control solutions are ready for use.
- Use **100 µL** of the prepared control solution in the same manner as a cow milk sample during testing (refer to Testing procedure).

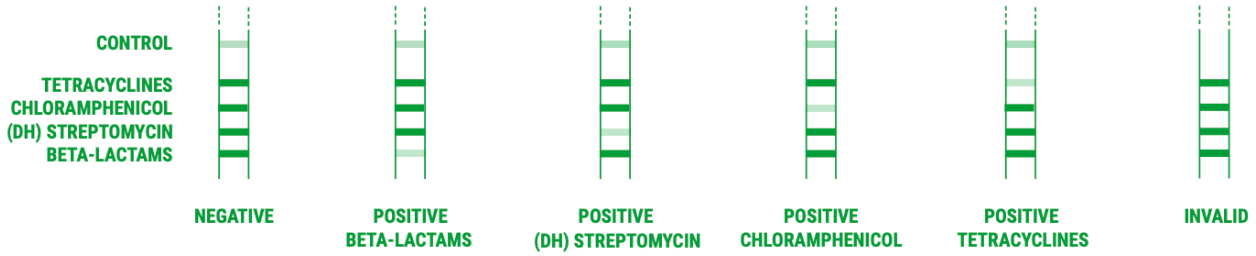
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6. Result Interpretation

- Visual interpretation
- Instrumental Interpretation: Using ReadSensor 2 / ANKAR-100

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6.1. Visual interpretation



Verify the presence of the upper control line.

- If the control line is not visible, the test result is **invalid** and no interpretation must be performed or continued.
- If the control line is visible, interpret the four test lines as follows: evaluate each test line individually by comparing the color intensity of the test line with that of the control line.

Begin, for example, with the bottom line corresponding to **ß-lactam antibiotics**.
If the test line is **darker than the control line**, the result is **NEGATIVE**. This indicates that, considering the sensitivity of the test, the milk sample contains no antibiotics or antibiotic residues below the detection limits stated in the enclosed limits of detection.

If the test line is **lighter than or similar to the control line**, the result is **POSITIVE**. This indicates that, considering the sensitivity of the test, the milk sample contains antibiotic residues at or above the detection limits stated in the enclosed limits of detection.

After interpreting one test line, repeat the same procedure for the remaining test lines.

- Record the interpretation result for each dipstick.
- Dipsticks may be archived as a permanent record, if required, by allowing them to dry completely before storage.

Line color intensity may increase during drying.

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6.2. Instrumental Interpretation: Using ReadSensor 2 / ANKAR-100

Reading Method

- Ensure that the latest available version of the reading method has been installed on the reader.
- Check the Unisensor website to confirm that the appropriate and most recent version is in use.

Reading

Refer to the **ReadSensor 2 / ANKAR-100 – APP088 User Manual** for the detailed reading procedure.

- If the control line is not visible (regardless of the presence or absence of test lines), consider the test result as **invalid**. Do not interpret the result and immediately perform a new test. The reader will confirm this condition by displaying an **INVALID** result.
- The dipstick must be read within **5 minutes** after completion of the test.

Based on the ratio calculated between the color intensities of the test line and the control line, the reader interprets the result as follows:

	Result Interpretation	
Test result:	Negative (NEG)	Positive (POS)
Interpretation:	Contamination absent or below the limit of detection	Contamination at or above the limit of detection

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7. Limits of detection

FAMILY	SUBFAMILY	COMPOUNDS DETECTED	SENSITIVITY ppb
B-LACTAMS	Penicillins	Ampicillin	2-2.5
		Penicillin G	0.5-1
		Amoxicillin	2-2.5
		Oxacillin	4-9
		Cloxacillin	2-4
		Dicloxacillin	2-4
		Nafcillin	10-55
		Piperacillin	0.5-3
		Ticarcillin	7-18
		Aspoxicillin	0.5-2
		Penicillin V	1-3
		Hetacillin	see AMPI
		Penethamate	see Pen G
	Cephalosporin	Ceftiofur	2.5- 7.5
		Desfuroylceftiofur	15-30
		Cefquinome	4-8
		Cefazolin	5-10
		Cephapirin	2-4
		Desacetylcephapirin	6-12
		Cefacetril	7-10
		Cefoperazone	1-2
		Cefalexin	600-1000
		Cephalonium	1-3

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FAMILY	SUBFAMILY	COMPOUNDS DETECTED	SENSITIVITY ppb
		Cephalotin	1-4
		Ceftriaxone	2-5
		Ceftizoxime	100-300
		Cefotaxime	9-18
		Cefuroxime	45-90
TETRACYCLINES	Tetracyclines	Tetracycline	3-5
		4-Epimer of Tetracycline	8-10
		Oxytetracycline	2-4
		4-Epimer of Oxytetracycline	10-11
		Chlortetracycline	6-8
		4-Epimer of Chlortetracycline	12-14
		Doxycycline	4-6
OTHERS	–	Chloramphenicol (Levomycetin)	0.1-0.15
		Chloramphenicol succinate	0.1-0.3
		Streptomycin	70-90
		Dihydrostreptomycin	70-90

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