



## Lateral Flow Test Kit

for the detection of  $\beta$ -lactams, tetracyclines and sulfonamides in cow, sheep and goat milk

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A.

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

**Use only the current version of Product Data Sheet enclosed with the kit.**

Symmetric BTS, S1248/S1296, is a lateral flow test for the simultaneous detection of  $\beta$ -lactams, tetracyclines and sulfonamides in cow, sheep and goat milk. This kit contains all reagents required for 48 or 96 reactions.

### Matrices:

Raw milk (cow, sheep, goat), pasteurized milk, milk powder, whey.

- Test time: 5min
- Shelf life: 12 months
- Storage: 2-8°C

## 1. Description

Symmetric BTS is a Lateral Flow test for the simultaneous detection of  $\beta$ -lactams, tetracyclines and sulfonamides in cow, sheep and goat milk.

## 2. Principle of the method

The Symmetric BTS lateral flow test is based on the competitive format immunoassay principle. The wells of the microtiter strips contain a  $\beta$ -lactam receptor, tetracycline and sulfonamide specific antibodies conjugated to colloidal gold. Milk samples are added into the wells. A dipstick with four capture lines, three test and one control, is dipped into the suspended mixture. A valid test should always have the upper control line red. The other three capture lines, one for  $\beta$ -lactams, one for tetracyclines and one for sulfonamides, are placed below the control line, respectively. If the sample is free of antibiotics, a color development occurs at the three test lines, indicating the absence of the targeted analytes in the milk sample. On the contrary, the presence of antibiotics in the sample will cause a reduced colored signal at the test lines depending on the class of antibiotics present.

## 3. Reagents provided

Symmetric BTS kit contains sufficient reagents and materials for 48/96 measurements.

- 6/12 containers each with 1 strip of 8 reagent microwells and 8 dipsticks.
- Positive standards
- Negative standards
- Instruction manual

## 4. Materials required but not provided

- 100 or 200 $\mu$ L adjustable single channel micropipettes with disposable tips
- One-touch Incubator (capable of maintaining a temperature at  $40\pm 2^\circ\text{C}$ )
- S-Flow software along with matching scanner device provided by lateral logic ltd

## 5. Storage instructions

Store kit components between  $2 - 8^\circ\text{C}$ . Do not freeze any components provided. Reseal the unused strips in the storing tube together with the desiccant bag provided. The expiry date of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated due to prior handling. Do not interchange individual components between kits of different lot numbers.

## 6. Safety and precautions for use

All reagents should be brought to room temperature ( $21 - 25^\circ\text{C}$ ) before use (at least half an hour) and covered when not in use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross contamination.

## 7. Negative and positive standards reconstitution

Symmetric BTS kit contains 1 strip of 8 microwells with negative standards (green) and 1 strip of 8 microwells with positive standards (red).

- **Negative (green):** Add 200 $\mu$ l distilled water into the microwell and mix well.
- **Positive (red):** Add 200 $\mu$ l **negative raw cow's milk** into the microwell and mix well.

After reconstitution follow the procedure as described in paragraph 10.

## 8. Sample preparation

**Milk :** Use 100 $\mu$ l of each milk sample directly in the immunoassay.

**Milk Powder :** Reconstitute the milk powder according to manufacturer's instructions. After reconstitution use 100 $\mu$ l directly in the immunoassay.

## 9. Sensitivity

Table 1. Limits of Detection for every compound.

	Compound	LOD ( $\mu\text{g}/\text{kg}$ )	Compound	LOD ( $\mu\text{g}/\text{kg}$ )
$\beta$ -Lactams	Penicillin-G	2 - 3	Cephapirin	6 - 10
	Ampicillin	2 - 4	Cefazolin	40 - 45
	Amoxicillin	2 - 4	Cephalexin	500 - 600
	Oxacillin	6 - 12	Cefalonium	4 - 8
	Cloxacillin	6 - 12	Ceftiofur	100 - 150
	Dicloxacillin	4 - 10	Cefquinome	6 - 10
	Nafcillin	8 - 12	Cefoperazone	4 - 8
	-	-	Cefacetile	8 - 12
Tetracyclines	Tetracycline	40 - 50	Chlorotetracycline	40 - 50
	Oxytetracycline	35 - 50	Doxycycline	50 - 70
Sulfonamides	Sulfapyridine	50 - 70	Sulfachloropyridazine	6 - 10
	Sulfathiazole	100 - 150	Sulfaguinidine	200 - 300
	Sulfamethoxazole	90 - 100	Sulfamethizole	25 - 35
	Sulfamethazine	2 - 8	Sulfadoxine	80 - 100
	Sulfamethoxyipyridazine	12 - 20	Sulfadiazine	4 - 8
	Sulfadimethoxine	4 - 8	Sulfamonomethoxine	2 - 6
	Sulfacetamide	250 - 300	Sulfaquinoxaline	6 - 10
	Sulfamerazine	4 - 8	Sulfasalazine	200 - 300

## 10. Method procedure

1. Plug in the One-touch Incubator and wait until the temperature has been stabilized at  $40^\circ\text{C}$ .
2. Before opening the reagents, take the kit out of the fridge (at least for half an hour) and wait until the temperature of the reagents reaches the ambient temperature.
3. Open one plastic pot and take out as many test strips and microwells as the number of milk samples to be tested. If needed, using scissors, carefully cut the number of reaction wells.
4. The pot with dipsticks should always be well closed after reagents have been taken out. - A pot with dipsticks should be emptied before another is opened.
5. Shake the milk sample(s) vigorously or vortex.
6. Place the microwell(s) in the incubator.
7. Place a new tip on the micropipette and dispense 100 $\mu$ l of milk into each of the microwells.

Using the same pipette tip, aspirate the sample up and down about 10 times to completely mix the lyophilized gold particles in the milk, while avoiding bubbles. The sample should turn into a uniform pink color. After mixing the particles, remove and discard the pipet tip. In case of more than 3 samples, an 8 channel multipipette should be used.

The ideal temperature of the milk sample is between  $4$  and  $18^\circ\text{C}$ .

8. Immediately, place the appropriate number of sticks into the wells.
9. Push the START(RUN) button. The 5-minute countdown starts.
10. When the 5 minutes of incubation are over, i.e. after the sound-signal, press START (STOP)\* again to stop the ringing tone and take the dipsticks out of the microwells.

11. Hold the dipstick from the colorful pad and remove the white cotton pad with your hands. Do not use a paper towel or any other material.

12. Place the dipstick inside the plastic holder in order to be scanned. In case of S-Flow scanner, the sticks must be facing up. In case of EPSON scanner, the sticks must be facing down (inverted) and the colored side must be facing the orange sticker.

13. Use S-flow software to interpret results as soon as possible and no later than 1 minute after the end of analysis. The software will use the ratio, R, of the test line and the control line to calculate the results (Table 2).

Table 2. Instrumental Interpretation

Ratio	R>1.1	0.9≤R≤1.1	R<0.9
Interpretation	Negative	Weak Positive	Positive

## Method procedure with pre-incubation

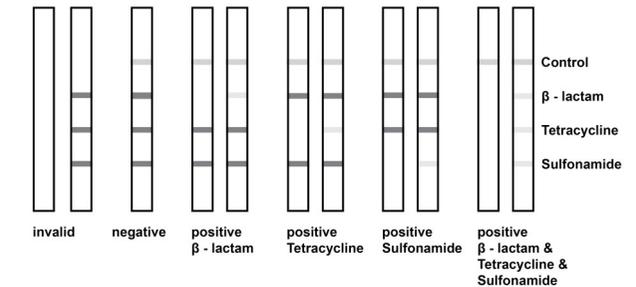
Alternative, you may use the Symmetric BTS kit with pre-incubation of the sample, especially with sheep and goat milk or samples with high fat content. Follow the procedure as described in paragraph 10. At **step 7** incubate the mixture of the sample and the lyophilized gold particles for **5 minutes**. Continue with step 8.

**Note:** In the case of **why** samples, result interpretation must be performed using the reader when the analysis completes 5 minutes; Quantum Read or visual interpretation is not applicable for this matrix.

## 11. Visual interpretation

When the test time is completed (5 min), the dipstick can also be visually read and interpreted according to the following figure.

Visual result interpretation index



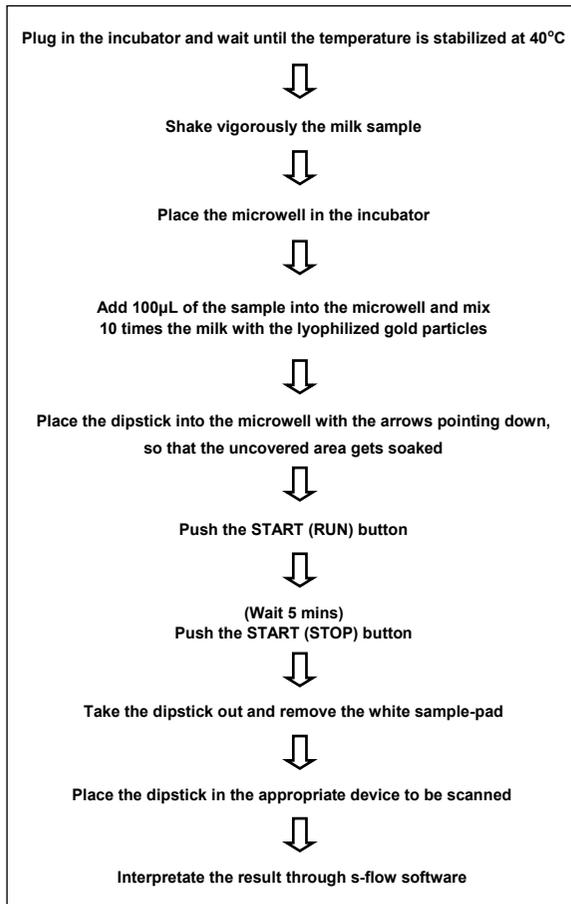
1. The control line should always be visible, if not the test is invalid.
2. When the control line can be seen, compare the intensity of each test line with the intensity of the control line:
  - i. If the test line is darker than the control line ( $T>C$ ), the sample contains no antibiotics or antibiotics at a lower level than the detection limits.
  - ii. If the test line is the same with the control line ( $T=C$ ), the sample contains antibiotics close to the detection limits
  - iii. If the test line is lighter than the control line ( $T<C$ ), the sample contains antibiotics above the detection limits.

## 12. Interferences

There are no interferences from somatic cells at  $10^6$  SCC/ml, bacteria at  $3 \times 10^6$  CFU/ml, fat or protein content.

## 13. Method summary

Total method time: 5 minutes.



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All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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