

DOMINIQUE DUTSCHER SA

SYMMETRIC FLORFENICOL

Lateral Flow Test Kit

for the detection of florfenicol in raw milk and whey

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

ProGnosis Biotech S.A. is ISO 9001:2015 certified.

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

Symmetric Florfenicol, S1548/S1596, is a lateral flow test for the detection of florfenicol in raw milk and whey. This kit contains all reagents required for 48 or 96 reactions.

Matrices:

Raw milk (cow, sheep, goat) and whey.

- Test time: 5min
- Shelf life: 18 months
- Storage: 2-8°C

1. Description

The Symmetric Florfenicol is a Lateral Flow test for the detection of florfenicol in raw milk (cow, sheep, goat) and whey.

2. General information

Antibiotics are widely used for the treatment and prevention of various animal diseases, such as mastitis and other microbial infections. After treatment, antibiotic residues can remain biologically active and unaltered in the animal's milk. The consumption of these residues poses significant risks, including allergic reactions, carcinogenicity, mutagenicity, hepatotoxicity, and the development of antibiotic resistance. Florfenicol, a fluorinated analog of thiamphenicol, is a broad-spectrum synthetic antibiotic used in veterinary medicine to treat bacterial infections in livestock, aquaculture and poultry. Its use is strictly regulated, with Maximum Residue Limits (MRLs) defined in Commission Regulation (EU) 37/2010 for various food producing species. MRLs have been established for muscle, skin/fat, liver and kidney tissues. However, the use of florfenicol is prohibited in animals that produce eggs or milk for human consumption.

3. Principle of the method

The Symmetric Florfenicol lateral flow test is based on the competitive immunoassay principle. The wells of the microtiter strips contain a florfenicol-specific antibody conjugated to colloidal gold. Milk samples are added into the wells. A dipstick with two capture lines, one test and one control, is dipped into the suspended mixture. If the sample is free of the antibiotic, color development occurs at test line, indicating the absence of the targeted analyte in the milk sample. On the contrary, the presence of the antibiotic in the sample will cause a reduced colored signal at the test line. A valid test should always have the upper control line red.

4. Reagents provided

The Symmetric Florfenicol 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

5. Materials required but not provided

- 100 or 200µL adjustable single channel micropipettes with disposable tips
- One-touch Incubator (capable of maintaining a temperature at 40±2°C)
- S-Flow software along with matching scanner device provided by lateral logic ltd

6. Storage instructions

Store kit components between 2 - 8°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.

7. Safety and precautions for use

All reagents should be brought to room temperature (21 - 25°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.

8. Negative and positive standards reconstitution

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

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

After reconstitution follow the procedure as described in paragraph 11.

9. Sample preparation

Milk and whey: Use **100µl** of each milk sample directly in the immunoassay.

10. Sensitivity

Limit of detection (LOD):

- Raw milk: 0.1 - 0.2 µg/L
- Whey: 0.05 - 0.1 µg/L

11. Method procedure

1. Plug in the One-touch Incubator and wait until the temperature has been stabilized at 40°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 sample(s) vigorously or vortex.
6. Place the microwell(s) in the incubator.
7. Place a new tip on the micropipette and dispense **100µl** of sample into the microwell. Using the same pipette tip, aspirate the sample up and down about 10 times to completely mix the lyophilized gold particles in the sample, 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.
8. Immediately, place the test strip(s) into the microwell(s).
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 test strip(s) out of the microwell(s).
11. Hold the test strip 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 test strip inside the plastic holder in order to be scanned. In case of S-Flow or 3PR scanner, the test strips 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).

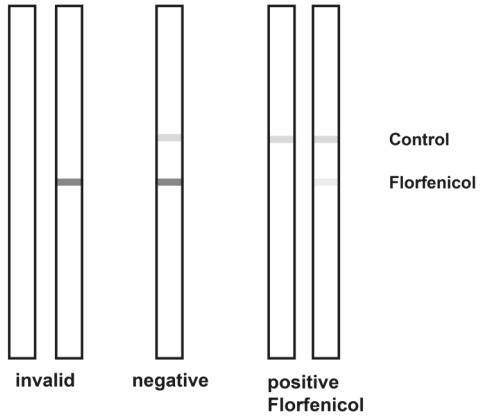
Table 2. Instrumental Interpretation

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

12. 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 the 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.

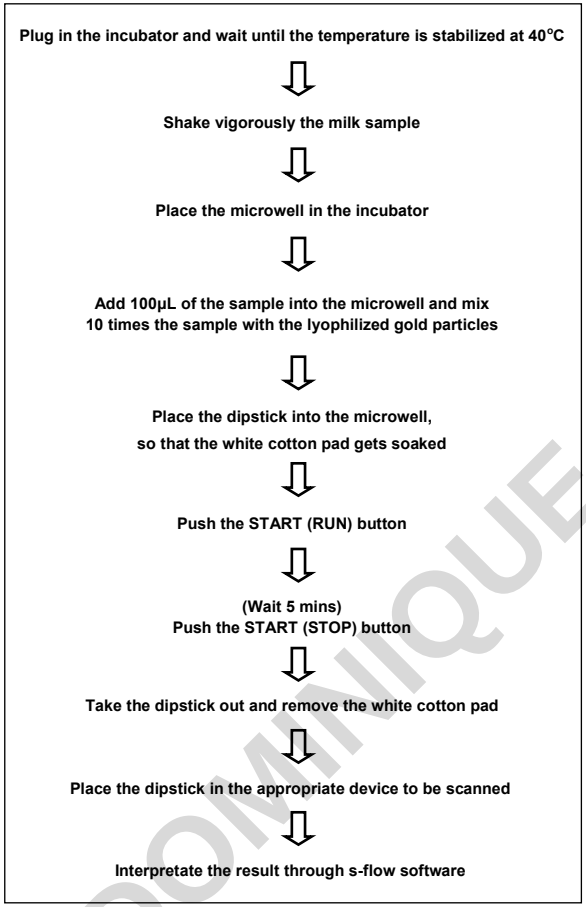
13. Interferences

There are no interferences from somatic cells at 10^6 SCC/ml, bacteria at $3x10^6$ CFU/ml, fat or protein content.

DOMINIQUE DUTSCHER SA

14. Method summary

Total method time: 5 minutes.



DOMINIQUE DUTSCHER SA

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.

ProGnosis Biotech S.A. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. ProGnosis Biotech S.A. shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product. This method is considered to be a screening method, before a legal action, samples detected as positives must be confirmed with a confirmation method. This product is meant to be used only For Research or Manufacturing use and by qualified technicians.

SYMMETRIC FLORFENICOL

LATERAL FLOW TEST KIT

for the detection of florfenicol in raw milk and whey.

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

ProGnosis Biotech S.A. is ISO 9001:2015 certified.

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

Symmetric Florfenicol, S1548/S1596, is a lateral flow test kit for the detection of florfenicol in raw milk and whey. This kit contains all reagents required for 48 or 96 reactions.

Matrices:

Raw milk (cow, sheep, goat) and whey.

- Test time: 5min
- Shelf life: 18 months
- Storage: 2-8°C

www.prognosis-biotech.com
e: exports@prognosis-biotech.com
t: +30 2410 623922
Farsalon 153 | 41335 Larissa, Greece

PROGNOSIS BIOTECH