



### **Lateral Flow Test Kit**

for the quantitative determination of Aflatoxin M1 in cow, sheep, goat and UHT 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 M1, S2148/S2196, is a Lateral Flow Test kit for the quantitative determination of Aflatoxin M1 in cow, sheep, goat, UHT milk and lyophilized milk.

This kit contains all reagents required for 48 or 96 reactions.

- Test Time : 10min
- Range: 0 - 150 ppt
- Limit of detection (LOD): 2ppt
- Shelf life: 12 months

This is an electronic version, please verify always the last one included in the kit.

## 1. Description

Symmetric M1 is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of Aflatoxin M1 in cow, sheep, goat, UHT milk and lyophilized milk.

## 2. General Information

Aflatoxins are toxic metabolites of major concern to the dairy industry, generally produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. They can have immunosuppressive, mutagenic, teratogenic and carcinogenic effects. Aflatoxins that are ingested by animals in contaminated pellets and forage are bio-transformed at the hepatic level into Aflatoxin M1 (AFM1). Aflatoxin is then excreted in this form into the milk used for human consumption and, it is also present in dairy products. AFM1 in milk and milk products is considered to pose certain hygienic risks for human health and as a result there is an established EU limit of 0.05 µg/kg (50 ppt).

## 3. Principle of the Method

The quantitative lateral flow test is based on the immunochromatography assay principles. The wells of the microtiter strips contain AFM1 specific antibodies conjugated to colloidal gold. Cow, sheep or goat milk samples are added into the wells and the suspended mixture is incubated. During this step, AFM1 (if it is present) binds to the antibodies. A dipstick with two lines, capture (test) and control, is dipped into the mixture. The liquid starts flowing vertically on the dipstick and passes through the two lines. A valid test should always have the upper control line red. If the sample is free of AFM1, a color development occurs at the test line, indicating the absence of AFM1 in the milk sample. On the contrary, the presence of AFM1 in the sample will cause a reduced colored signal at the test line. The test line color intensity is indirectly proportionate to the concentration of AFM1 present in the samples. By utilizing S-Flow software AFM1 is accurately quantified.

## 4. Reagents Provided

Symmetric M1 kit contains sufficient reagents and materials for 48/96 measurements.

- 6/12 containers each with 1 strip of 8 reagent microwells and 8 dipsticks.

## 5. Materials required but not provided

- 200 or 300 µl adjustable single channel micropipettes with disposable tips
- **S-Flow** software along with matching scanner device provided by lateral logic ltd
- **One-touch** Incubator for strips with automatic release

## 6. Storage Instructions

Store kit components between 2 - 8°C (36 - 46°F). 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 (18 - 24°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. Method Procedure

1. Place the equipment of automatic release (test top) on the incubator and connect it to the appropriate input, located at the left side of the incubator. 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 container and take out as many dipsticks and microwells as milk samples to be tested (tests per experiment ≤ reader positions). If needed, using scissors, carefully cut the number of reaction wells.
4. The container with dipsticks should always be well closed after reagents have been taken out. - A container with dipsticks should be emptied before another is opened.
5. Shake the milk samples vigorously or vortex.
6. Place the microwell(s) in the incubator
7. Place a new tip on the micropipette and dispense 200µl of milk into each of the microwells. Using the same pipet 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° C.**

8. Push the START(RUN) button. The 5-minute countdown starts.
9. Place the appropriate number of sticks into the automatic release equipment.
10. When the 5 minutes are over, the stick will be automatically dropped into the microwells and the **second incubation** of 5 minutes will start. **Always check if the sticks have been dropped into the wells.**
11. When the 5 minutes of the second 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.

12. Remove the white cotton sample-pad of the stick. Touch the stick with your hand from the colorful pad and remove the white pad with your hands. Do not use a paper towel or any other material.
13. Place the stick inside the plastic holder inverted in order to be scanned. The colored side must be facing the orange sticker.
14. Use S-flow software to quantify results as soon as possible and no later than 10 minutes after the end of analysis. Choose the type of milk analyzed. For pasteurized and fresh cow milk choose Cow. For fresh goat and fresh sheep milk choose Goat and Sheep, respectively. For Ultra High Temperature milk and Lyophilized milk choose UHT and Lyophilized, respectively. The software will use a Lot specific curve to calculate the results in parts per trillion (ppt). Refer to the S-Flow manual for a detailed description of the procedure. A simple visual interpretation of the stick is NOT possible.

## 9. General Specifications

- **Cross-reactivity:** The anti-Aflatoxin M1 antibody has <5% cross reactivity with Aflatoxin M2 and no cross-reactions other mycotoxins (Ochratoxin A, Zearalenone, Deoxynivalenol and Fumonisin B1) and other unrelated compounds, such as antibiotics (Benzylpenicillin, Cefalonium, Oxytetracycline, Erythromycin, Neomycin, Enrofloxacin, Sulfadiazine, Trimethoprim and Dapsone).
- **Matrices:** Raw and pasteurized cow, goat and sheep milk, UHT milk and lyophilized milk.
- **IC50** = 22.5 - 50ppt.
- **Limit of detection:** 2ppt.
- **Limit of quantification:** 8ppt.
- Accuracy of the results (concentrations between 30 and 75ppt of AFM1) < 8% CV
- Quadruplicate value at 50ppt CV (%) < 7
- Positive/negative cut off at 50 ng AFM1/L, >44.2 ppt

## 10. Interferences

There are no interferences from somatic cells at 10<sup>6</sup> SCC/ml or bacteria at 3x10<sup>6</sup> CFU/ml.

## 11. Performance Evaluation

### Proficiency Tests

All products participate frequently in Proficiency Tests. For more information, visit the individual product page in our website: [www.prognosis-biotech.com](http://www.prognosis-biotech.com)

## 12. Summary of method

Total method time: 10 minutes

Plug in the incubator and wait until the temperature is stabilized

at 40°C



Shake vigorously the milk samples



Place the microwells in the incubator



Dispense 200µL of each sample into the microwells and mix  
10 times the milk with the lyophilized gold particles



Push the START (RUN) button



Place the test sticks into the automatic release equipment



(Wait 5 mins)

The sticks will be automatically dropped



(Wait 5 mins)

Take the stick out and remove the white sample-pad



Place the strip in the appropriate scanner to be scanned



Quantify through s-flow software



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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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