VERSION N2
CAT.NUMBER: S3124/S3148

SYMMETRIC B1 ES

LATERAL FLOW TEST KIT

for the quantitative determination of Aflatoxin B1 in grains, cereals, nuts and animal feed

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 B1 ES, S3124/S3148, is a Lateral Flow Test kit for the quantitative determination of Aflatoxin B1 in grains, cereals, nuts and animal feed.

This kit contains all reagents required for 24 or 48 reactions.

Matrices:

Type I: Almond, Barley, Brown rice, Buckwheat, Cashews, Corn, Corn flakes, Corn flour, Corn pastone, Cottonseed, Dried figs, Hazelnut, Mug beans, Oats, Pasta, Peanut, Pistachio, Roasted chickpeas, Sesame, Silage, Sorghum, Soybeans, Soy flour, Sunflower seeds, Wheat, Wheat flour, White rice.

Type II: Animal feed, Corn Gluten Meal, DDGS

- Sample preparation: extraction
- Test time (reaction time after samples and reagents preparation): 10min
- Range: 0 - 20ppb
- Shelf life: 12 months
- Storage: 2-8^{o}C

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

Specifications

- The LOD of the method is: 0.5ppb (Type I), 0.6ppb (Type II)
- The LOQ of the method is: 0.7ppb (Type I), 1ppb (Type II)
- Cross-reactivity: The cross-reaction of the anti-Aflatoxin antibody with Aflatoxin B1, B2, G1 and G2 is 100, 43, 47 and 8% respectively.

1. Description

Symmetric B1 ES is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of Aflatoxin B1 (AFB1) in grains, cereals, nuts and animal feed.

2. General Information

Aflatoxins are toxic metabolites of major concern to the food industry, generally produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. They can have immunosuppressive, mutagenic, teratogenic and carcinogenic effects. Aflatoxins can be present in grains, spices, cereals and other commodities associated with human food or animal feed. Crops may be contaminated with aflatoxins. AFB1 is the most toxic and frequently detected form. The other types (B2, G1 and G2) present a significant danger if the concentration is at a high level. Animals are exposed to aflatoxins by consumption of feed that have fungal strains producing aflatoxins during growth, harvest or storage. Symptoms of toxicity in animals range from death to chronic diseases, reproductive interference, immune suppression, decreased milk and egg production. Most controlling government agencies worldwide have regulations regarding the amount of aflatoxins allowable in human and animal foodstuffs. Accurate and rapid determination of aflatoxin presence in commodities is of paramount importance.

3. Principle of the Method

The quantitative lateral flow test is based on the immunochromatography assay principles. The wells of the microtiter strips contain AFB1 specific antibodies conjugated to colloidal gold. Diluted extract is added into the wells. A dipstick with two capture lines, test and control, is dipped into the well. The suspended mixture 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 AFB1, a color development occurs at the test line, indicating the absence of AFB1 in the sample. On the contrary, the presence of AFB1 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 AFB1 present in the samples. By utilizing S-Flow software and the symmetric quantification technology [1, 2], AFB1 is accurately quantified.

4. Reagents Provided

Symmetric B1 ES kit contains sufficient reagents and materials for 24/48 measurements.

Reagents (Store at 2-8ºC)	Quantity for 24 wells	Quantity for 48 wells
Pots each with 1 strip of 8 reagent microwells	3	6
Sample Diluent Tubes	24	48
High Range Solution	1	1

5. Materials required but not provided

- A grinder sufficient to render sample to particle size of fine instant coffee
- Balance with 0 - 50g measuring capability and Graduated cylinder - 50ml
- Ethanol (9.75ml reagent grade per sample) and Deionized water
- Filter Paper Whatman #1 or equivalent, Filter Funnel and Miscellaneous laboratory plastic or glass tubes 5 - 15ml
- Tube roller or Vortex mixer
- 100 - 300µl adjustable micropipettes (single or multi channel) with disposable tips
- **S-Flow** software along with matching scanner device

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. Preparation of the extraction solvent

1. Measure 35ml of distilled or deionized water and transfer it into a glass bottle.
2. Measure 65ml of ethanol (reagent grade) and transfer it into the bottle containing the water.
3. The quantity of extraction solvent (100ml of 65% ethanol) is sufficient for 6 samples (5gr each). A user may prepare smaller or larger amounts of the extraction solvent maintaining the ratio of 6.5 parts ethanol to 3.5 parts distilled or deionized water by volume. Prepare a new extraction solvent prior to use

9. Sample preparation

1. The sample must be collected according to established sampling techniques. Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
2. Weigh out a 5g ground portion of the sample and add 15ml of the **65% ethanol** (Prepare 65% Ethanol by adding 5.25ml of distilled or deionized water to 9.75ml of ethanol (reagent grade) for each sample to be tested. Mix using a tube roller for 5 minutes (or vortex for 2min). **The ratio of sample to Extraction Solution is 1:3 (w/v).**
3. Allow the particulate matter to settle. Filter the extract through a Whatman #1 filter paper (or equivalent) and collect the filtrate. Alternatively, centrifuge 1ml of the extract for 2min using a mini centrifuge (spin).
4. Add **200µl** of filtrate (or supernatant) into the Sample Diluent Tube provided and mix well. Run the diluted filtrate within 30 minutes.

NOTE 1: The extracted sample should have pH value of 6.2 - 7.0. If the pH is less than 6.2, the pH should be neutralized using NaOH.

NOTE 2: If a range 0 - 100ppb is required, mix the diluted sample with **High Range Solution** 1:4 (five times). Then, use only the **5X Dilution** Matrix Type.

10. Method Procedure

1. Before opening the reagents, take the kit out of the fridge and wait until the temperature of the reagents reaches the ambient temperature.
2. Open one plastic pot and take out as many test strips and microwells as samples to be tested.
3. The pot with dipsticks should always be well closed after reagents have been taken out.
4. Dispense 200µl of diluted filtrate into the microwell and pipette up and down 4 times to completely mix the lyophilized gold particles in the sample, while avoiding bubbles. The sample should turn into a uniform pink color. In case of more than 2 samples, an 8 channel multipipette should be used.
5. Place the appropriate number of sticks into microwells **immediately** and set timer for 10 minutes
6. When the 10 minutes are over, take the dipsticks out of the microwells and remove the white cotton sample-pad of the stick **immediately**. 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.
7. Place the stick inside the plastic holder in order to be scanned. In case of EPSON scanner, the sticks must be facing down (inverted) and the colored side must be facing the orange sticker. **NOTE**: The sticks should be scanned within 2 minutes after the sample-pad removal.
8. The software will use a Lot specific curve to calculate the results (ppb) according to the matrix sample type. Refer to the S-Flow manual for a detailed description of the procedure. A simple visual interpretation of the stick is NOT possible

11. Performance Evaluation

11.1 Reference Materials

Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at info@prognosis-biotech.com.

11.2 Proficiency Tests

All products participate frequently in Proficiency Tests. For more information, visit the individual product page in our website: **www.prognosis-biotech.com**

11. Method Summary

Total method time: 10 minutes

Extract the samples

⇩

Add 200µl of extract into the Sample diluent tube provided

⇩

Dispense 200µl of each sample into the microwells and mix 5 times the sample with the lyophilized gold particles

⇩

Place the appropriate number of sticks into microwells immediately.

⇩

(Wait 10 mins)
Take the stick out and remove the white sample-pad immediately

⇩

Place the stick in the appropriate device to be scanned

⇩

Quantify through s-flow software

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