

تمقیق*اتی م*واد شیمیایی و مولکولاربیولوژی

# Aflatoxin B1 Screening Test in Food & Feed (RAPID TEST)

# **BACKGROUND:**

Aflatoxins are produced as secondary metabolites by the fungus Aspergillus flavus and A. parasiticus on variety of food products. They are potent toxic, carcinogenic, mutagenic, immunosuppressive and have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. There are four major aflatoxins, B1, B2, G1, G2 plus two additional metabolic products, M1 and M2, that are of significance as direct contaminants of foods and feeds. Aflatoxin B1 (AFB1) is normally predominant in amount in cultures as well as in food products. Food products contaminated with aflatoxins include cereal, oilseeds, spices, tree nuts and milk. Regarding the amount of aflatoxins allowable in human and animal foodstuffs, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed. Thus it is very important to develop an rapid and accurate detection tool for detecting the presence of aflatoxin in commodities.

# **INTENDED USE:**

Aflatoxin B1 Test is a qualitative immunochromatographic assay for the detection of aflatoxin B1 in grains, nuts, oilseeds, cereals, spices and other commodities which are necessary to messure due to high matrix effect.

# PRINCIPLE:

The basis of the immunochromatographic assay in test card is an antigen-antibody reaction. A specific antibody against aflatoxin recognizes the aflatoxin molecules in the samples. The results are read visually by observing the development of coloured bands. The control band (control line) is not influenced by aflatoxin in the sample and should be present in all cases in order to prove the test card is valid. The test band (test line) is not visible in the absence of aflatoxin in the sample and if aflatoxin is present in concentrations of 5 ppb\* and higher the band is clearly visible.

\*Different detection concentrations can be designed according to your need.

# MATERIALS PROVIDED:

- 10 aluminum foil pouches each containing Af B1 test card with a dropper and a desiccant
- 10 Sample collection tubes containing the assay diluent
- 10 centrifugal tube for use
- One instruction for use

### MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Minitype disintegrator or Grinder sufficient to render sample to particle size of fine instant coffee
- 2. Balance of 0.1g sensibility
- 3. Glassware: Erlenmeyer flask, beaker, filter funnel, graduated cylinder, cuvette, burette, pipettor
- 4. Filter paper, sop paper

# **PRECAUTIONS:**

- 1) For best results, strict adherence to these instructions is required.
- 2) All specimens should be handled as being potentially poisonous.
- 3) Do not open or remove test kit from their individually sealed pouches until immediately before their use
- 4) Do not reuse test kit.
- 5) All reagents must be at room temperature before running the assay.
- 6) Do not use reagents beyond the stated expiration date marked on the package label.
- 7) The components in this kit have been quality control tested as standard batch unit. Do not mix components from different lot numbers.

# STORAGE INSTRUCTIONS

The kit can be stored at room temperature (2-30°C) or refrigerated. The test kit is stable through the expiration date marked on the package label. DO NOT FREEZE. Do not store the test kit in direct sunlight.

Email: Betageneco@gmail.com



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#### **EXTRACTION PROCEDURE:**

grain (corn, wheat, barley, rye, oats, rice, millet, canola), soy flour, nuts (peanut, hazelnut, almond, Brazil nut, Macadamia nut), pistachios, coconut flour, sunflower seeds, figs, dates and cashew nuts

Note: The sample must be collected according to established sampling techniques

- 1). Grind a representative sample to the particle size of fine instant coffee (50% passes through a 20 mesh screen).
- 2). Weigh out a 2.0g ground portion of the sample into a centrifugal tube.
- 3). Add 4mL of water and 4mL of ethyl acetate into the centrifugal tube. Shake sufficiently for about 5 minutes. Keep static or centrifugalize in 3000rpm. Collect 2 mL of supernatant liquid into a small beaker. Filtrate if needed.
- 4). Dry the filtrate by cool wind. Then dissolve the dried component on the bottom of the beaker with 0.6 mL of the assay dilution.

*ATTENTION:* The designed quantity of assay dilution is to detect aflatoxin B1 at 5 ppb and above. If you want to detect aflatoxin B1 at a higher permissible dose, please add distilled water .

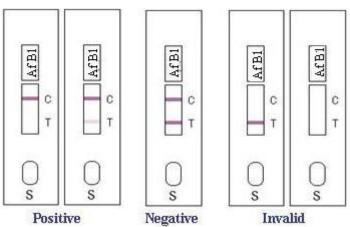
5). The sample is now ready. The extraction should be diluted according to the national practical residue limit. NOTE: If the sample is oilseed such as groundnut, the sample should be primely dealed with 8 mL of hexane and 2 mL of water. Shack sufficiently and keep static. Discard the oil layer (up layer) and process the step 3).

# **TEST PROCEDURE:**

- 1) Please carefully read the instruction prior to testing. Bring all the reagents to room temperature before use.
- 2) Remove the test card from the foil pouch. Place it horizontally on the desk. Please use it soon in one hour.
- 3) Drop 3(three) drops of the sample into "S" hole and start to time.
- 4) Interpret test results at about 8 minutes. Do not interpret after 12 minutes.

# **INTERPRETATION OF RESULTS:**

The presence of only one clear band in zone "C" indicates a positive result. If one vague band appears in zone "T" lighter than the band in zone "C", the result is also considered positive. The presence of two clear color bands ("T" and "C") within the result window indicates a negative result. If controll band is not visible in zone "C", the result is **considered invalid** 



# LIMITATION

The Quicking Aflatoxin Test is a useful tool offering a rapid and accurate testing in practical detection, exceeding with its convenience. The result is usually considered as a primery estimation. Other testing methods should be followed to give a comprehensive estimation if necessary.

Email: Betageneco@gmail.com