Agitest™ Food Safety Rapid Test Strip Instruction Manual – Aflatoxin Total

1 Brief Information

Aflatoxins are a group of highly toxic secondary metabolic products of some Aspergillus spp. These metabolites, aflatoxin B_1 , B_2 , G_1 , and G_2 , are all carcinogenic and mutagenic to humans and animals. Among which, aflatoxin B_1 is the most prevalent and toxic. Foods and feeds are susceptible to aflatoxin contamination during production, processing, transportation and storage. The United State Food and Drug Administration (FDA) sets the maximum level of total aflatoxins for all foods as 20 ng/g (20 ppb).

AgitestTM Food Safety Rapid Test - Aflatoxin total is an immuno-chromatographic test for the detection of aflatoxin B_1 , B_2 , G_1 , and G_2 in foods and feeds. Results are interpreted visually.

* Aflatoxins have been confirmed as a group 1 carcinogenic compound (IARC, 2012).

*Detection Limit:

5 ppb of Aflatoxin B₁ standard in 80% methanol solution.

2 Intended use

Agitest[™] Food Safety Rapid Test - Aflatoxin Total is for the qualitative detection of aflatoxin B_1 , B_2 , G_1 , and G_2 in foods and feeds. The test should be used for the detection of small amounts of aflatoxins. This test is proper for food quality control in food processing plants specifically with inbound goods, processing stages and final products.

3 Reagents Provided

Each test kit contains:

3.1 Rapid test alu-pouch
3.2 Buffer
3.3 Instruction manual
50 pouches
12 mL, 1 bottle
1 manual

4 Equipment/materials required

- 4.1 $20-200~\mu L$ and 1,000 μL Pipetmans.
- 4.2 Accurate weighing scale.
- 4.3 Homogenizer or grinder.
- 4.4 1.5 mL Microcentrifuge tubes.
- 4.5 15 mL & 50 mL Centrifuge tubes.
- 4.6 Vortex mixer.
- 4.7 Shaker.
- 4.8 Timer.
- 4.9 Methanol (\geq 99.8%).
- 4.10 Clean water.

5 Storage Instructions

5.1 Store the test kit between 2 to 30°C.

- 5.2 Keep away from direct sunlight.
- 5.3 Do not freeze.

6 Cautions

- 6.1 Do not test your sample with Aflatoxin Total Strip only. The test will not react.
- 6.2 This product is for food testing and *in vitro* diagnostic use only, not for human use.
- 6.3 This product contains non-toxic buffer. To maintain test accuracy, please keep bottle upright to prevent leakage.
- 6.4 This product is designed as a single-use test. Please do not re-use test strips.
- 6.5 This product is designed for screening purpose only. If analysis of ingredients is required, please send the sample to your local laboratory for further analysis.
- 6.6 To ensure test accuracy, please do not use expired test strips.
- 6.7 All samples require extraction (80% methanol solution) and dilution (buffer). Please do not test the sample directly with the test strip.
- 6.8 This rapid test strip product may not be able to apply to test aflatoxins content in some kinds of food samples, such as coffee beans.

7 Preparation of samples

- 7.1 Initial notes
 - 7.1.1 Any powder, dust and polluted devices may affect test results. In order to avoid contamination in the operating procedure, please clean all utensils and operating environment with 75% ethanol before obtaining the sample. Please wear gloves during operation to ensure the detection accuracy.
 - 7.1.2 The samples should be homogenized or milled before testing.
 - 7.1.3 To prepare 80% methanol solution: mix 40 mL methanol with 10 mL clean water (4:1).
- 7.2 Sample Preparation
 - 7.2.1 Weigh 2 g of homogenized or milled sample to a 15 mL centrifuge tube.
 - 7.2.2 Add 4 mL 80% methanol solution and vortex/ mix for 2 minutes until whole sample are completely immersed.
 - In order to satisfy different detection limits, dilute 2 g sample with different volume of 80% methanol according to below table.

80% Methanol	4 mL	6 mL	8 mL
Sensitivity	10 ppb	15 ppb	20 ppb

- 7.2.3 Leave sample mixture to settle for 2 minutes.
- 7.2.4 Pipette 40 μ L sample mixture supernatant into a 1.5 mL microcentrifuge tube with 160 μ L buffer.

8 Test Procedure

- 8.1 Open the alu-pouch and take out Aflatoxin Total Strip and Release Pad (please handle the colored sticker portion only and do not touch the reaction zone). Shown as Figure A.
- 8.2 Insert Release Pad into the 1.5 mL microcentrifuge tube with sample prepared from <u>7.2</u>. The arrow on the release pad must be downward (liquid level shall not over the arrow). Mix the reagents and the sample mixture together (the sample mixture will turn light pink) for 30 seconds. Leave Release Pad in the tube and wait for 2 minutes at room temperature. Shown as Figure B.
- 8.3 Remove Release Pad (if significant residuals can be seen, repeat <u>8.2</u>) and insert Aflatoxin Total Strip into the 1.5 mL microcentrifuge tube, wait for 15 minutes, then read the results.

Figure A.

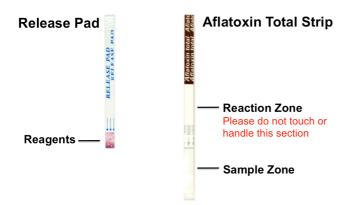
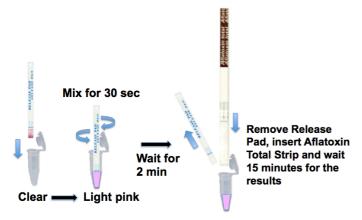


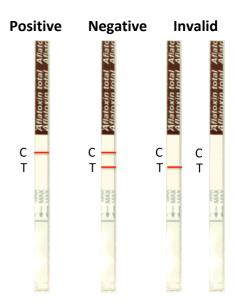
Figure B.



Note! If you use Aflatoxin Total Strip only, the test will not be able to react.

9 Results and sensitivity

- 9.1 **Positive result: one colored band** (the Control line) is visible within the reaction zone.
- 9.2 **Negative result: two colored bands** (Control line and Test line) are visible within the reaction zone.
- 9.3 Invalid result: no colored band or only Test line is visible within the reaction zone, the test is considered invalid. Please check if the alu-pouch is damaged or damp, the sample concentration is too high or the reaction time is over/under 15 minutes. Please re-test with a new test strip.



➤ Wait 15 minutes for the results. Please read the results immediately without further manipulation.

REGA BIOTECHNOLOGY INC. www.regabio.com Page 2