NATIONAL FOOD SAFETY FOUNDATION

RAPID TESTING KITS

DETECTION OF AFLATOXIN M1 RESIDUES IN MILK AND MILK PRODUCTS USING AFLATOXIN M1 RAPID TESTING KIT
INTRODUCTION

Aflatoxin is a potent liver toxin known to cause cancer in animals. It was found that in swine, aflatoxin can cause reduced weight gain, reduced ability to resist diseases, hepatitis and death. The Food and Drug Administration (FDA) has established action levels of 20 parts per billion (ppb) for grain and feed products, and 0.5 ppb for milk. Grain, feed, or milk containing aflatoxin at or above these levels cannot be sold for food or feed in inter-state sales.

The Rapid Aflatoxin M1 Test is a qualitative one-step competitive inhibition immunoassay for the detection of aflatoxin M1 in milk and milk products. It detects the presence of aflatoxin M1 at 0.3 μg/kg or higher in milk samples by utilizing highly specific reactions between antibodies and aflatoxin M1 in milk samples. It only takes approx. 20~25 min.

Advantages of the Aflatoxin M1 testing Kit

- It is rapid; results obtained in 7 minutes
- It is highly sensitive; adapt to specific regulations (EU and US)
- It is easy to use; no extra devices required; easily perform on site or in lab
- It is simple to use; visual interpretation of results
- It is accurate and reliable
- It is cost effective
- Cost effective

PRINCIPLE OF THE TEST

The Aflatoxin M1 Test Strip consists of a membrane strip containing an Aflatoxin M1 conjugate. A Control Line, produced by a different antibody-antigen reaction, is also present on the membrane strip. The microtiter wells contain colloidal gold labeled antibodies, which are preincubated with the milk sample or extract.

The colloidal gold labeled antibodies move with the milk sample or extract by capillary action along the membrane. In the absence of Aflatoxin M1 in the milk sample or extract, the colloidal gold labeled antibody contacts the immobilized Aflatoxin M1 conjugate on the strip.

An antibody-antigen reaction occurs forming a visible line. The Control Line is not influenced by the presence or absence of Aflatoxin M1 in the milk sample or extract, and therefore, should
be present in all reactions. The formation of two visible lines indicates a negative result (below the detection limit or cut-off).

If Aflatoxin M1 is present in the milk sample or extract, it competes with the immobilized Aflatoxin M1 conjugate in the test area for binding sites on the colloidal gold labeled antibody. If a sufficient amount of Aflatoxin M1 is present in the milk sample or extract, it will fill all of the available binding sites, thus preventing attachment of the labeled antibody to the immobilized Aflatoxin M1 conjugate and, therefore no line will develop. If a colored line is not visible in the Test Line region, or if the Test Line is significantly lighter than the Control Line, the milk sample or extract is positive (above the detection limit or cut-off).

**CONTENTS OF THE AFLATOXIN M1 TEST KIT**

1. One operational Instruction.
2. Microtiter wells coated with colloidal gold labeled antibodies, in a resealable aluminum pouch
3. Strip test cassette in a sealed pouch
4. Droppers in a resealable bag
5. Sample Buffer, must be diluted 1:10 with deionized water before use

**ADDITIONAL REAGENTS AND MATERIALS (NOT PROVIDED)**

1. Timer
2. Dry Milk Dilution Buffer (provided upon request)
3. Vortex mixer

**SENSITIVITY**

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Limit of Detection (ppb)</th>
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<tr>
<td>Aflatoxin M1</td>
<td>0.2-0.3 0r 0.3-0.4</td>
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**Sample Preparation**

1. Milk - Adjust to room temperature. Proceed to Strip Test Procedure,
2. Infant Formula (liquid) – Adjust to room temperature. Proceed to Strip Test Procedure,
3. Dry Milk Powder – Prepare according to package directions using the Dry Milk Dilution Buffer instead of water. Cap tightly and vortex until the dry milk powder dissolves. Proceed to Strip Test Procedure

4. Infant Formula (powder) – Prepare according to package directions using deionized or distilled water. Cap tightly and vortex until the infant formula powder dissolves. Proceed to Strip Test Procedure,

**STRIP TEST PROCEDURE**

1. Prepare sample according to Sample Preparation instructions
2. Open the pouch containing the microtiter well strips. Remove the required number of wells (1 per sample) and place in holder. Place the remaining wells in the pouch and reseal.
3. Open the resealable bag containing the droppers. Remove one dropper per sample or extract and label with the sample id. Note: One dropper is used for each sample or extract for the entire procedure. Do not discard the dropper until the test is complete. Use only the appropriate labeled dropper for the appropriate sample or extract as the use of droppers from other samples or extracts will result in contamination and produce inaccurate results.
4. Open the required number of pouches containing the strip test cassette (1 per sample or extract). Remove the cassette being careful not to touch the membrane of the strip test. Label one cassette for each sample or extract.
5. Using the appropriate dropper, place 6 drops (about 200 µL) of the sample or extract into the microtiter well. Incubate for 2 minutes at room temperature.
6. Using the appropriate dropper, carefully mix the sample or extract in the microtiter well by drawing the sample or extract up into the dropper and expelling into the well repeatedly until the reagent coated in the well is dissolved. Incubate for 2 minutes at room temperature.
7. Using the appropriate dropper, transfer the entire contents from the microtiter well to the sample collection region of the test strip cassette. Incubate for 10 minutes at room temperature.
8. Interpret the results within 5 minutes.

**INTERPRETATION OF RESULTS**
Negative:

Two lines are visible and the Test Line (T) is the same as or darker than the Control Line (C), which also is the Reference Line (R). This indicates that the sample is negative.

Positive:

No purplish red band appears in T line indicating that the sample is positive.

Invalid:

Reference Line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for an invalid result. Review the procedure and re-peat the test with a new test device. Stop using the test kit immediately if the problem is not solved and contact your local distributor.

STORAGE AND SHELF LIFE

Store at 15-25°C, out of sunlight and moisture, maintain sealed, do not freeze or use beyond the expiration date. The shelf life is 12 months.

PRECAUTIONS

1. Do not use after the expiration date.

2. The test device should remain in the sealed pouch until use.

3. Use device as soon as possible but within 1 hour after removal from the pouch specially.
4. Do not touch the white membrane in the mid of the test device.

5. Use the plastic dropper for one time in case cross reaction happens

6. It may lead into wrong result if there is bleaching or oxidation.

7. Do the test at room temperature. It takes longer time at high temperature, and shorter time at low temperature.

ORDERING INFORMATION

In case you want to place your order for this kit or you want more information about our Testing Kit products, you can reach us by calling +256 393 100655/+256 772475784 or visit our website; www.foodsafetyltd.com. You can also email us on foodsafety@foodsafetyltd.com or visit our office which is located in Kulambiro-Ntinda on Plot 1099, Block 215, Kondogolo Zone, Off Ntinda Kisaasi Road on Kulambiro Ring road near East High School Ntinda, Kampala-Uganda