

CHAPTER 11

MYCO✓ TEST KIT

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## 11.1 GENERAL INFORMATION

The Myco✓ test is a competitive enzyme-linked immunosorbent assay that provides quantitative measurement for the presence of aflatoxin in select grains and commodities.

**The test kit is limited to providing aflatoxin measurements between 5 – 80 ppb.**

Accurate aflatoxin measurements above 80 ppb can be obtained by performing a supplemental analysis involving a diluted extract.

## 11.2 PREPARATION OF SOLUTIONS

### a. Extraction Solution.

The extraction solvent used in the Myco✓ test method is a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (ACS grade or better) and 30 percent water.

- (1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.
- (2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.
- (3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.
- (4) Store this solution at room temperature in a tightly closed container until needed.

**NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.**

### b. Wash Solution.

- (1) Transfer the contents of the Wash Concentrate vial to a 500-ml plastic squeeze bottle and add 475 ml of distilled or deionized water.
- (2) Swirl to mix.

### 11.3 EXTRACTION PROCEDURES

- a. Place a sheet of filter paper (Whatman #1 folded or equivalent) into a funnel mounted over a clean collection container.
- b. Label the collection container with the sample identification.
- c. Transfer 50 grams of ground sample into an extraction mixing jar.
- d. Add 250 ml of the (70/30) methanol/water extraction solvent.
- e. Cover the extraction jar and blend on high speed for 2 minutes.
- f. Allow the extract to stand for 2-3 minutes to allow the slurry to settle.
- g. Filter a minimum of 15 ml of the extract into the collection container.

### 11.4 TEST PROCEDURES

- a. Allow reagents, antibody-coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test.
- b. Place the appropriate number of red mixing wells and clear test wells into a microwell holder.

**NOTE: The maximum number of test samples that can be run at one time is 19. Using a strip of 12 wells, designate 5 wells for the calibrators and the remainder of the wells for test samples.**

- c. Using a pipette, dispense 150  $\mu$ l of Enzyme Conjugate into each red mixing well.
- d. Dispense 50  $\mu$ l of each calibrator and sample into the appropriate red mixing wells using an adjustable or fixed 50  $\mu$ l pipette.

**NOTE: Use a clean pipette tip for each addition.**

mixing wells	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
	O	O	O	O	O	O	O	O	O	O	O	O
	C0	C10	C20	C40	C80	S1	S2	S3	S4	S5	S6	S7

Where C0 is the zero control, C10 is the 10 ppb control, C20 is the 20 ppb control, C40 is the 40 ppb control, and C80 is the 80 ppb control. S1 is sample 1, S2 is sample 2, S3 is sample 3, etc.

- e. Using a multi-channel pipette, mix the contents of the wells by repeatedly filling and emptying the tips into the mixing wells.
- f. Using a multi-channel pipette, transfer 100 µl of each reaction mixture directly into the corresponding clear test wells. Discard the mixing wells into an appropriate waste container.
- g. Let the reaction mixture incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.
- h. At the end of the 5-minute incubation period, dump the contents of the wells into an appropriate waste container. Using a 500-ml squeeze bottle containing wash solution, vigorously wash each well by overfilling. Repeat the vigorous wash for **a total of four washes**.
- i. After the last wash, invert the wells and tap on absorbent paper to remove residual wash solution. Wipe excess liquid from the bottom of the wells.
- j. Pour substrate solution into a clean reagent reservoir.
- k. Dispense 100 µl of substrate solution into each test well using a multi-channel pipette.
- l. Let the substrate solution incubate for **exactly 5 minutes**. Mix the solution in the wells by gently swirling the plate on a flat surface for the first 15 seconds.
- m. Pour stop solution into a clean reagent reservoir.

- n. Dispense 100 µl of stop solution into each test well using a multi-channel pipette.
- o. Read and record the optical density of the wells at 650 nm using a Hyperion MicroReader™ 3 well reader. Make sure that the well bottoms are clean and dry before placing in the reader. Read the test results within 20 minutes of test completion. Use the data reduction software provided by SDI to quantify results.

## 11.5 REPORTING AND CERTIFYING TEST RESULTS

- a. Report all results on the pan ticket and the inspection log to the nearest whole ppb.
- b. Sample results over 80 ppb are reported as >80 ppb unless a supplemental analysis is performed.
- c. Refer to the Certification section of the handbook for more detailed certification procedures.

## 11.6 SUPPLEMENTAL ANALYSIS

- a. Diluting the Sample Extract.

If quantitative results are above the testing limits (i.e., 80 ppb) of the test kit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 80 ppb, the sample extract must be diluted so that a value between 5 and 80 ppb is obtained.

The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

- b. Example.

If the original analysis reported the aflatoxin value at 100 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

- (1) Dilute 5 ml of the original extract with 5 ml of the extraction solvent mixture. The total volume is 10 ml. This is a 1 to 2 dilution (compares volume in the beginning with the total volume in the end).

- (2) Multiply the analytical results obtained by 2 to obtain the actual aflatoxin concentration. For example, if 54 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 108 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

$$\begin{aligned}\text{True Aflatoxin Value} &= (10 \div 5) \times 54 \text{ ppb} \\ &= 2 \times 54 \text{ ppb} = 108 \text{ ppb}\end{aligned}$$

## 11.7 CLEANING LABWARE

a. Negative Tests ( $\leq 20$  ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests ( $> 20$  ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour the liquid down the drain and place the materials in a garbage bag and discard.

## 11.8 WASTE DISPOSAL

a. Negative Results ( $\leq 20$  ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results ( $> 20$  ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

## 11.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits

- (1) 48 antibody-coated microtiter wells.
- (2) 48 red-marked mixing wells.
- (3) 5 vials each containing 2 ml of 0, 10, 20, 40, and 80 ppb of aflatoxin calibrators.
- (4) 1 vial containing 8 ml of aflatoxin-HRP enzyme conjugate.
- (5) 1 vial containing 8 ml of substrate.

- (6) 1 vial containing 8 ml of stop solution.
- (7) 1 vial containing 25ml of 20X wash concentrate.
- (8) 4 multi-channel pipette reservoirs.

b. Materials Required but not Provided.

- (1) Methanol - ACS grade or better.
- (2) Deionized or distilled water.
- (3) 100 ml graduated cylinder.
- (4) Whatman #1 filter paper or equivalent.
- (5) Glassware with 125 ml capacity for sample extraction.
- (6) Filter funnel.
- (7) 50  $\mu$ l pipette with disposable tips.
- (8) 50 -200  $\mu$ l multi-channel pipette.
- (9) 500 ml plastic squeeze bottle.
- (10) Blender with mixing jars.
- (11) Balance.
- (12) Sample grinder.
- (13) Hyperion MicroReader™ 3 Model 4027-002 with 650 nm filter.
- (14) Timer.
- (15) Waterproof marker.
- (16) Microwell holder.

## **11.10 STORAGE CONDITIONS**

- a. Store test kits between 36°- 46° F when not in use. Avoid prolonged storage of kits at room temperature. Do not freeze test kits.
- b. Do not use reagents from other SDI aflatoxin kits with different lot numbers.
- c. Bring kits up to room temperature 64°- 86° F prior to use.
- d. Do not use kit components beyond their expiration date.