

ENVIROLOGIX

Application Guide

Testing for Cry9C in corn meal, flour and grits, using
EnviroLogix QuantiPlate Kit for Cry9C (Cat. No. AP 008)

Background:

The EnviroLogix QuantiPlate Kit for Cry9C is designed for the detection of this Bt endotoxin in bulk corn grain. This Application Guide describes the sample extraction and preparation steps required to use this kit to detect Cry9C endotoxin (found in StarLink and other Cry9C genetically modified corn varieties) in processed corn products (cornmeal, corn flour and corn grits). Read the entire kit Product Insert before using this Application Guide.

Note: A negative test with this kit is not an indicator of the absence of other genetic modifications.

Sample Preparation and Extraction:

This protocol calls for a small sample to be analyzed. It is essential that this sample be well mixed and representative of the larger bulk that it is drawn from. Thorough mixing and determination of an appropriate sampling plan are critical to the results of this testing, and are the responsibility of the user of this test kit.

1. Cornmeal and corn flour samples are ready to extract without further treatment. Corn grits should be ground to the consistency of cornmeal, using the Waring blender and Mason jars described in the product insert.
2. Weigh 5 grams of cornmeal, flour or ground grits into a 50 mL capacity tube or vial. Note: larger sample sizes may be used; just be sure to maintain the ratio of extraction solution to weight of sample described in the next paragraph. If you require additional 20x Grain Extraction Concentrate, please contact EnviroLogix for Cat# KR 001.
3. To cornmeal and ground corn grits, add **7.5 mL/gram of 1x Grain Extraction Solution** (37.5 mL to 5 grams). Add **10 mL/gram of 1x Grain Extraction Solution** to corn flour samples (50 mL to 5 grams). Mix vigorously and allow to extract for 1 hour. Mix again at the end of the 1 hour.
4. An aliquot of the extracted sample must be clarified by centrifugation at 5000 x g for 5 minutes.

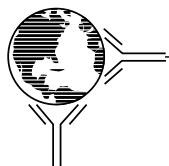
Sample Extract Dilution:

Concentrations of Cry9C endotoxin in StarLink corn can vary greatly. To screen products for presence or absence of Cry9C modification, use undiluted clarified extracts in the High Sensitivity Protocol for maximum sensitivity. If sample extracts produce more color than the highest calibrator, and you wish to quantitatively determine the Cry9C endotoxin in that sample, you will have to dilute the sample extract in 1x Grain Extraction Solution and run it in the Rapid Protocol. Multiple dilutions may be required to get the sample extract within the range of calibration. Failure to sufficiently dilute positive extracts can result in erroneously low Cry9C concentrations.

Assay Protocol and Interpretation:

1. Pipet 100 μ L of clarified sample extract into the ELISA microplate as directed in the Product Insert under "How to Run the Assay", in the "High Sensitivity Protocol" section of the Product Insert. Continue with the High Sensitivity Protocol instructions as stated in the Product Insert.
2. To interpret a qualitative screening assay, any sample extracts that produce lower OD results than that of the lowest calibrator in the assay (0.01% StarLink/0.2 ppb Cry9C) are presumed to be negative for Cry9C modification. Cry9C-modified corn products containing 0.01% StarLink or more should result in OD's greater than that of the lowest Calibrator.
3. For quantification of Cry9C endotoxin, follow the Product Insert instructions for graphing Cry9C Calibrator (%StarLink or ppb Cry9C levels) vs. OD, and interpolating each sample's StarLink/Cry9C concentration from the curve. Multiply the ppb Cry9C results from the curve by the dilution incurred during extraction (7.5 for meal and grits, 10 for flour). If you are using the %StarLink calibrator values, do not multiply by this extraction buffer to sample weight ratio. If you made any post-extraction dilutions, you must multiply both the ppb Cry9C and the %StarLink by this factor.

Technical Assistance:



ENVIROLOGIX INC.

500 Riverside Industrial Parkway, Portland, ME 04103

Phone: 207-797-0300 • Fax: 207-797-7533 • Email: info@envirologix.com

www.envirologix.com

Rev. 03/10/04