StarLink Corn in Corn Flour and Corn Meal—ELISA Method

First approval October 17, 2001

Objective

Cry9C protein in StarLink (SL) corn is an endotoxin produced from a gene derived from *Bacillus thuringiensis (Bt)*. This method is a quantitative enzyme-linked immunosorbent assay (ELISA) determination of *Bt*-modified corn in corn flour and meal. Proprietary antibodies, from Strategic Diagnostics Inc., specific for Cry9C protein are used. The method is calibrated to estimate the weight percent of modified corn in corn flour and corn meal.

Apparatus

1. Centrifuge tubes, 15-ml polypropylene, with conical bottom.
2. Sample cups, 125 ml (4 oz).
3. Transfer pipets, polypropylene.
4. Plastic tape, for manual plate washing.
5. Wash bottle.
6. Precision pipets, capable of delivering 100–1000 µl.
7. Class A pipet, 32 ml.
8. Vortex mixer.
9. Balance, capable of 0.01-g measurement.
10. Centrifuge, capable of 3000–5000 rpm.
11. Microtiter plate reader, capable of reading absorbance at 450 nm, preferably with subtraction of 650-nm reading.
12. Multichannel pipette, 100 µl.
13. Reagent reservoirs for multichannel dispensing.
14. Automated plate washer.
15. Test tube rack, for 15-ml centrifuge tubes.
16. Graduated cylinder, 100 ml.

Reagents

Items 1–16 are available as GMO ✓ Bt9 Maize Kit from Strategic Diagnostics Inc. See Note 1.

1. Buffer concentrate (20×). Store at 2–8°C when not in use.
2. Coated strip wells (eight strips) and strip holder.
3. Plate sealers.
4. *Bt*9 maize conjugate. Store at 2–8°C when not in use.
5. Color solution. Store at 2–8°C when not in use.
6. Stop solution. Store at 2–8°C when not in use.
7. *Bt*9 Cry9C corn reference standards at 0, 0.0075, 0.025, and 0.10% SL.
8. Buffer solution. Dilute 1 part buffer concentrate (reagent 1) plus 19 parts deionized water. Mix well.

http://dx.doi.org/10.1094/AACCIntMethod-11-20.01

StarLink Corn in Corn Flour and Corn Meal—ELISA Method (continued)

Procedure

Preparation of coated strip wells

Remove coated strip wells and holder from foil bag. Return unneeded strip wells to bag and reseal. Secure desired number of test strip wells in strip holder, allowing two wells per sample and standard.

Sample extraction

1. Weigh 4.0 0.1 g corn flour or 20.0 0.1 g corn meal into a 125-ml (4-oz) sample cup.
2. Add 32.0 ml of buffer solution (reagent 8) to flour samples or 100 ml of buffer solution to each meal sample. Shake for at least 1 min.
3. Allow the cups to stand for 10 min; then shake again for at least 1 min.
4. Repeat step 3 twice more for a total of 30 min.
5. Pour off supernatant from each sample into a 15-ml conical centrifuge tube. Centrifuge at 3000–5000 × *g* for 5 min.

ELISA assay

1. Add 100 µl of *Bt*9 maize conjugate (reagent 4) to each well of the coated strip wells of prepared plate (reagent 2). Pipet 100 µl of each standard (reagent 7) and sample extract into duplicate wells. Cover with supplied plate sealers (reagent 3) to prevent contamination and evaporation.
2. Incubate strips at room temperature for 1 hr.
3. Wash five times with buffer solution (reagent 8, 300 µl per well). If automated plate washer is not available, manual washing can be performed as follows:
    a. Invert strip holder and discard contents of plate into a sink or suitable waste container. Tap inverted strip holder onto a stack of paper towels.
    b. Fill each well with an overflow volume of buffer solution (reagent 8).
    c. Invert strip holder to discard contents. Tap inverted strip onto paper towels.
    d. Repeat steps b and c four times.
    e. Do not let wells dry out.
4. Add 100 µl of color solution (reagent 5) to each well. Incubate for 10 min at room temperature.
5. Add 100 µl of stop solution (reagent 6) to each well, in the order of addition of color solution in step 4.
6. Read optical density (OD) of each cell using microtiter plate reader at 450 nm. Set reader to zero (blank) on air. Read wells within 15 min after adding stop solution (reagent 6).

StarLink Corn in Corn Flour and Corn Meal—ELISA Method (continued)

Calculations

Perform quadratic least squares regression of eight calibration data points to obtain calibration equation in the form:

$$\%SL = a(OD)^2 + b(OD) + c$$

where *a–c* are least squares coefficients. Calculate %SL for all sample wells. Report average of two wells as the result.

A run is accepted if *all* of the following criteria are met:

a. Average OD of 0% SL standard is 0.20.
b. Average OD of 0.1% SL standard is 1.0.
c. Coefficient of variation of pairs of OD of 0.0075, 0.025, and 0.10% SL standards is 15%.
d. The r^2 statistic for the regression is 0.980.

Notes

Use these general precautions.

1. Reagents must be stored at 2–8° when not in use.
2. Allow reagents to reach ambient temperature (18–27°) before beginning test.
3. Do not freeze kit components or expose to temperatures greater than 37°.
4. Kit is intended for one-time use only. Do not reuse wells containing sample, reagents, or wash solution.
5. Do not use reagents past stated expiration date.
6. Cry9C corn extracts can be stored refrigerated for up to 2 days.
7. Do not expose color solution (reagent 5) to direct sunlight.
8. Do not mix reagents from different kit lots.
9. Do not dilute or adulterate test reagents or use samples not validated for the test procedure. This may give inaccurate results.

References

1. Lipp, M., Anklam, E., and Stave, J. 2000. Validation of an immunoassay for the detection and quantification of Roundup-Ready soybeans in food and food fractions. J. AOAC Int. 83:919-927.
2. Stave, J. W., Magin, K., Schimmel, H., Lawruk, T., Wehling, P., and Bridges, A. 2000. AACC collaborative study of a protein method for detection of genetically modified corn. Cereal Foods World 45:497-501.
3. U.S. Department of Agriculture, Grain Inspection, Packers and Stockyards Administration. GIPSA Directive 9181.2 (Jan. 10, 2001), Performance verification of rapid tests for the detection of biotech events. Website: www.usda.gov/gipsa