ABRAXIS® Glyphosate in Rapeseed
Sample Preparation for Strip Test

1. Intended Use
For the detection of Glyphosate in rapeseed.

2. Sensitivity
10 ppm in matrix

3. Materials and Reagents Required
Analytical balance
50 mL or greater volume size of plastic/glass bottles or vials
Serological pipette or graduated cylinder
Microcentrifuge tubes, 2.0 mL
Microcentrifuge device capable of ≥8000 x g
Disposable pipettes (optional)
Micropipettes with disposable plastic tips (optional)
Vortex mixer (optional)
Timer
Rotator and/or shaker
Blender or grinder device (i.e. food processor, coffee bean grinder, etc.) Optional: Grinder, IKA Tube Mill control PN 500104. Used in conjunction with disposable grinding tubes, MT 40 PN 500105
Deionized or distilled water
ABRAXIS® Glyphosate Strip Test (PN 500095 [20T]; PN 500098 [5T])

4. Notes and Precautions
This procedure is intended for use with rapeseed. Samples should be ground into a powder-like consistency using a blender or grinder device (i.e. food processor, coffee bean grinder, etc.). If using an IKA grinder, pour maximum of 20 grams of sample into the disposable grinder tube and grind in an IKA Tube Mill control grinder set at 25,000 (twenty-five thousand) rpm for 60 seconds. If the sample accretes at the sides of the vessel of the chosen grinder, dislodge the sample and grind at a lower speed, and slowly increase to maximum until the sample accretes again. Repeat until all whole seeds appear to have been broken and the sample has a uniform grain size. Other matrices should be thoroughly validated before use with this procedure.

5. Sample Preparation Procedure

5.1 Weigh 2.0 g of sample powder into an appropriately labeled bottle or vial.
5.2 Add 40 mL of deionized or distilled water. Vortex or shake to mix.
5.3 Place sample bottle/vial onto rotator or shaker for 10 minutes.
5.4 Transfer 2 mL of extract to an appropriately labeled microcentrifuge vial.
5.5 Centrifuge for 5 minutes at ~8000 x g. Make sure the centrifuge is properly balanced.
5.6 The supernatant of the centrifuged sample is at 20-fold dilution. The sample must be diluted to a total 20,000-fold (twenty-thousand-fold) dilution before performing the assay. Below is the recommended method for dilution.
    5.6.1 Transfer 0.2 mL of supernatant to 19.8 mL of deionized or distilled water into an appropriately labeled vial, rotate to homogenize.

5.6.2 From the previous dilution (5.6.1), transfer 2 mL of supernatant to 18 mL of deionized or distilled water into an appropriately labeled vial, rotate to homogenize.

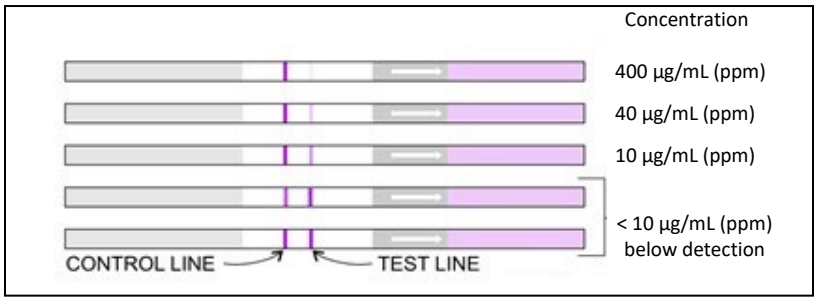
5.7 Proceed to Sections E (Test Preparation) and F (Testing of Samples) in the Glyphosate Strip Test Kit user's guide.

6. Evaluation of Results

Rapeseed sample concentration is determined by comparison of the intensity of the test line to the intensity of the control line on the same test strip. Although control line intensity may vary, a visible control line must be present for results to be considered valid. Test strips with a test line which is darker than or of equal intensity to the control line indicates a result which is below the limit of detection of the test. Test strips with a test line which is lighter than the control line indicates a result which is between 10 ppm and 400 ppm. Test strips with a very faint test line or no test line visible indicates a result which is > 400 ppm. Results should be determined within 5-10 minutes after completion of the strip test procedure. Determination made using strips which have dried for more or less than the required time may be inaccurate, as line intensities may vary with drying time.

Control Line	***Test Line***	***Interpretation***
No control line present	No test line present	Invalid result
Control line present	Very faint or no test line present	>400 µg/mL (ppm)
Control line present	Moderate intensity test line present	Between 10 and 400 µg/mL (ppm)

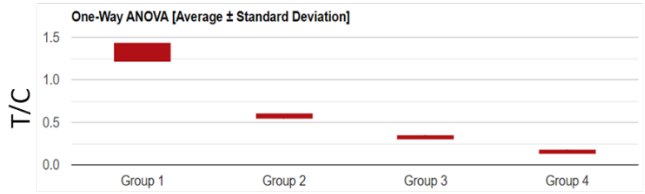
The appearance of test strips may also be compared to the illustration below to determine approximate sample concentration ranges. Please note that the illustration is intended for the demonstration of test line to control line intensity only. Results should not be determined by comparing the intensity of test lines from test strips to the test line intensity of the illustration, as the overall intensity of test strips may vary slightly with different lots of reagents. To obtain semi-quantitative results in the range of 0-400 ppm, solutions of known Glyphosate concentration (control solutions) must be tested concurrently with samples. Sample test line intensities can then be compared with control solution test line intensities, yielding approximate sample concentrations. Do not use strips run previously to determine semi-quantitative sample concentrations, as test line intensities may vary once strips are completely dry.



7. Performance Data

The Limit of Quantification (LOQ) values were determined by spiking glyphosate into a non-detect (<7.5 ppb by ELISA) residue matrix (to approximate these concentrations). Ten replicate test portions for each concentration were derivatized and then analyzed using the test strips.

Results:
All the blanks were non-detect. The low positive spike (10 ppm) samples were low positive, the medium positive spike (40 ppm) samples were medium positive, and high positive spike (400 ppm) samples were high positive. The ABRAXIS® Glyphosate Strip Test for rapeseed samples will detect 10 ppm or higher due to the 20,000-fold (twenty-thousand-fold) dilution required during sample preparation. At this level, the test line exhibits moderate intensity. At levels greater than 400 ppm, the test line is faint or not visible.



Per one-way ANOVA analysis, a statistically significant difference ($P<0.0001$) exists between the mean T/C values for the tested spike levels in the rapaseed sample.

Data Summary

Group	N	Mean T/C	STDEV T/C	%CV T/C
Non-detect	10	1.32	0.11	8.6
Low Positive	10	0.58	0.04	6.8
Medium Positive	10	0.34	0.02	6.8
High Positive	10	0.17	0.02	13.8

T - Test Line Intensity
C - Control Line Intensity
T/C - Concentration-Determining Ratio

8. Assistance

For ordering or technical assistance contact:

Gold Standard Diagnostics
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Horsham, PA 19044
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