Glyphosate in Green Coffee
Sample Preparation for Strip Test

1. Intended Use
For the detection of Glyphosate in Green Coffee.

2. Sensitivity
20 ppb in matrix

3. Materials and Reagents Required
Metal hammer or mallet (optional)
Disposable zipper seal plastic bags (4 MIL heavy duty) (optional)
Ika® Tube Mill control (part no. 0004180001) with individual disposable grinding chambers (MT40), Vitamix® Professional Series 750 blender, or equivalent (countertop coffee grinder, blender, etc.) (Optional)
Disposable spatulas, VWR part no. 80081-190 or equivalent (optional)
500 µm sieve, USA Standard #35 or equivalent (optional)
Analytical Balance
50 mL plastic tubes or equivalent
Microcentrifuge tubes, 2 mL
Microcentrifuge capable of 8,000 x g
Serological pipette or graduated cylinder
Deionized or distilled water
Rotator and/or shaker, GlasCol® Cat #099A RD4512 or equivalent
Micropipettes with disposable plastic tips (optional)
Vortex Mixer (optional)
4 mL glass vials (optional)
Timer
ABRAXIS® Glyphosate Strip Test [PN 500095 (20T); PN 500098 (5T)]

4. Notes and Precautions
This procedure is intended for use with green coffee bean samples. Other matrices should be thoroughly validated before using this procedure.

It is highly recommended for the samples be as finely ground as possible before extraction to produce accurate results. This may be done with an Ika® Tube Mill, Vitamix® Professional Series 750 blender, coffee grinder, or equivalent. Green coffee beans may need to be coarsely crushed with a hammer or mallet in a plastic bag prior to being placed in a grinding device so they will grind efficiently, as well as avoid damaging the device. It is recommended to do this when employing an Ika® Tube Mill for grinding. Refer to technical bulletin "Glyphosate in Green Coffee –Whole Green Coffee Beans Sample Grinding" for instructions. If desired, a 500 µm sieve can be used after grinding to ensure uniform sample consistency.

If processing multiple samples using the Ika® Tube Mill for which disposable grinding chambers were purchased, each sample must be ground in a new, clean individual disposable grinding chamber. Use of the same grinding chamber with multiple samples may result in sample contamination and produce inaccurate (biased high or biased low) sample results. If processing samples with an Ika® Tube Mill without disposable grinding chambers, or using an alternative method, such as a Vitamix® Professional Series 750 blender, coffee grinder, etc., the device

must be thoroughly washed then rinsed with deionized or distilled water between samples to prevent contamination which would produce inaccurate (biased high or biased low) sample results.

5. Sample Preparation Procedure

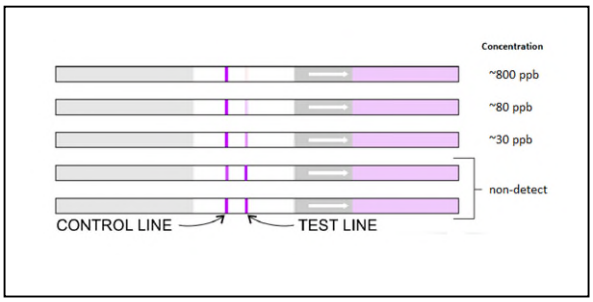
5.1 Weigh 0.5 g of sample powder into an appropriately labeled bottle or vial.
5.2 Add 20 mL of deionized or distilled water. Vortex or shake to mix.
5.3 Place sample bottle/vial onto rotator or shaker for 10 minutes.
5.4 Remove sample bottle/vial from the rotator or shaker. Let sample settle for 10 minutes.
5.5 Transfer 1.5 mL of extract to an appropriately labeled microcentrifuge vial.
5.6 Centrifuge for 5 minutes at ~8000 x g. Make sure the centrifuge is properly balanced.
5.7 Using the supernatant as the sample, being careful not to disturb the pellet formed at the bottom of the tube, immediately proceed to analyze with the Glyphosate Strip Test. Proceed to Sections E (Test Preparation) and F (Testing of Samples) in the Glyphosate Strip Test Kit user's guide,

6. Evaluation of Results

Green coffee 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 30 ppb and 800 ppb. Test strips with a very faint test line or no test line visible indicates a result which is > 800 ppb. 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	>800 ng/mL (ppb)
Control line present	Moderate intensity test line present	Between 30 and 800 ng/mL (ppb)

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 0-800 ppb, 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.



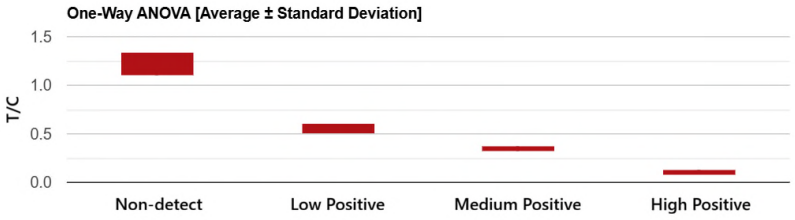
7. Performance Data

The ABRAXIS® Glyphosate Strip Test for Green Coffee samples will detect in the range of 20 ppb or higher due to the 40-fold dilution required during sample preparation. At this level, the test line exhibits moderate intensity. At levels greater than 800 ppb, the test line is faint or not visible.

The Limit of Quantification (LOQ) values were determined by spiking glyphosate into a non-detect (<20 ppb by ELISA, and <10ppb by LC-MS) residue matrix (to approximate these concentrations). Ten replicate test portions for each concentration were derivatized and then analyzed using the test strips.

Data Summary

Group	N	Mean T/C	STDEV T/C	%CV T/C
Non-detect	10	1.219	0.117	9.6
Low Positive	10	0.555	0.0521	9.4
Medium Positive	10	0.343	0.0295	8.6
High Positive	10	0.114	0.0165	14.4



T- Test Line Intensity
C - Control Line Intensity
T/C - Ratio (of T/C)

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 green coffee sample.

8. For ordering or technical assistance contact:

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Date this Technical Bulletin is effective: 06/09/2026

Version: 02