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Glufosinate ammonium (BASTA) Paint Assay for Screening for BAR Resistance Gene in Tobacco

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ABSTRACT

Glufosinate ammonium (BASTATM/LIBERTYTM) Paint Assay for Screening for the presence of the [BAR Resistance Gene](#) in Transgenic Tobacco.

PROTOCOL CITATION

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GUIDELINES

Use of herbicides at work in Illinois requires a [Commercial Illinois Department of Ag Pesticide Applicator License](#).

Temperature inversions will occur with glufosinate ammonium based herbicides in hot, dry conditions with a wind speed less than 3 mph or greater than 10 mph causing herbicide drift to unintended targets. Do not use in an enclosed, wind free environment such as a growth chamber if the chamber contains plants that you do not want to come in contact with glufosinate ammonium. Increase humidity and maintain an airflow between 3 and 10 mph in a greenhouse to ensure there is not drift.

Please note that this protocol is species specific. The concentration of glufosinate ammonium to generate a physiological response without systemically damaging the plant differs for each species.

MATERIALS TEXT

- Glufosinate ammonium based herbicide, [Liberty 280 SL](#)
- Q-tips
- 200 ul tips, [USA Scientific Inc 11110700](#)
- 2-200 ul pipette, Eppendorf, Research Plus
- Graduated cylinder, 50 mL
- Permanent marker, Sharpie

SAFETY WARNINGS

[Read the EPA pesticide safety label in its entirety.](#)

Anyone working around pesticides, even if they are not handling them directly should be trained annually on the [EPA Agricultural Worker Protection Standard](#).

Wear appropriate PPE as prescribed in the label.

BEFORE STARTING

The younger a seedling the more susceptible it will be to systemic damage by herbicides even if it contains the appropriate herbicide resistance gene. For best results, do not screen plants until they are at least 4 inches tall and contain multiple sets of true leaves.

Herbicide Dilution

1 Make a working stock of 1.5 g/L of glufosinate ammonium.

1.1 Determine the amount of glufosinate ammonium active ingredient by reading the EPA herbicide label.

[Liberty 280 SL](#) contains 280 g/L.

1.2 Calculate and perform the required dilution from the amount of active ingredient in the stock herbicide to obtain a working stock of 1.5 g/L.

Liberty 280 SL (280 g/L) will be diluted 160 ul Liberty 280 SL into 30 mL of distilled water.



Wear appropriate PPE as prescribed by the herbicide label at all times when handling the herbicide. Notify workers around you that you will be applying herbicide. Ensure that all workers who will be working in areas where herbicide is applied have been trained annually on the EPA Worker Protection Standard.

Herbicide Application

2 Draw a line across the upper surface of a healthy leaf with a permanent marker.



- 3 Apply the working stock (1.5 g/L glufosinate ammonium) herbicide to the section of the leaf from the tip to the permanent marker line.



Paint Assay Interpretation

- 4 After approximately 5 days, observe the leaf.
 - If the leaf has died to approximately the level of the marker line, the plant is glufosinate ammonium sensitive indicating that it most likely does not contain the BAR resistance gene.
 - If the leaf has died entirely or well past the marker line, the plant may be experiencing physiological distress (water stress, disease pressure, etc) unrelated to the paint assay. The assay is inconclusive. Correct the growth conditions to remove the plant stressor and repeat the assay on a healthy leaf.
 - If the leaf does not exhibit necrosis on the leaf from the tip to the marker line, the plant most likely contains the BAR resistance gene.