

Roundup Ready® Trait Purity: An indirect evaluation method to assess the percentage of seed or seedlings that contain the Roundup Ready (*cp4-epsps*) transgene in Roundup Ready varieties*

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PLANT CULTURE Greenhouse/Growth Chamber

Container..... Seed germination flats with drainage holes (e.g., 10 in. x 20 in. x 3 in. flat)
Media..... Peat-based, potting soil mix or other suitable fertile, well-drained germination media
Temp/Light..... 20 to 24 C; >16 hour day length
No. of Seedlings..... 200 per entry per replicate, minimum (e.g., ten - 10 in. rows/flat, 20-30 seedlings/row); exact count at full emergence, prior to herbicide application
No. of Replicates..... 4, minimum
Test Duration 4 to 6 wk total test duration from planting to scoring

HERBICIDE APPLICATION *For purposes of this standard test only*

Herbicide Rate, Formulation, and Application Apply a two-fold maximum field label rate of glyphosate as a foliar application (the two-fold rate is 3.78 lb of the active ingredient glyphosate). Apply the two-fold label rate twice (see below). Use only Monsanto brand Roundup® formulations labeled for field use on Roundup Ready® alfalfa and follow all label safety precautions and handling instructions. Use only distilled or deionized water to dilute.

Plant Stage At Application Seedlings should be treated twice before scoring with the first application occurring on 2nd trifoliolate stage plants and the second application 7 to 10 days after the first. This will assure that there are no escapes. Healthy, vigorous seedlings should be used in the test (i.e., approximately 2 to 3 wks old at the first application date).

Post Application Incubation and Culture Allow a minimum of 24 hours after each application before watering. Fertilize and water adequately to encourage vigorous seedling growth. Late germinating plants should be removed during the initial emergence count, or subsequently excluded from the scoring counts. Typically, seedlings will be ready to score 5 to 7 days after the second application depending upon growth conditions. Score when ≥99% of non-tolerant control seedlings demonstrate obvious glyphosate injury (normally this will be death).

SCORING Tolerance is evaluated when ≥99% non-tolerant check cultivar seedlings have been killed or have significant characteristic symptoms of Roundup injury.

Tolerant..... Roundup Ready® seedlings continue to grow and develop. If the glyphosate formulation applied contains surfactant, the still-growing Roundup Ready seedlings may also exhibit temporary chlorosis (“flashing”), necrotic flecking or leaf shape irregularities (see “Helpful Information”).

Non-tolerant..... Non-tolerant (susceptible) seedlings initially exhibit stem tip wilt and systemic chlorosis followed by total collapse, necrosis and death. Killed seedlings rapidly decompose and may become very difficult to see or count within a few days depending upon conditions.

CHECK CULTIVARS	Approximate Expected Tolerance (%)	Acceptable Range of Reaction (%)
<u>Tolerant (Roundup Ready®)</u>		
FGI-RR90 ^a	90	85-95
<u>Non-tolerant</u>		
Saranac ^b	0	0-1

Values for standards are percent of total plants in Tolerant Class rounded to the nearest whole number.

^a FGI-RR90 contains proprietary, patent protected and internationally regulated biotechnology-derived (transgenic) plants. As such, seed will only be made available to evaluators from FGI in observance of applicable government regulations. Prior to seed shipment, requestors must agree to use the seed exclusively for the testing purposes defined above; the FGI-RR90 materials transfer/use agreement is available from FGI (address below); no other agreement is required.

^b Any conventionally-bred (non-tolerant) check cultivar may be substituted at the discretion of the evaluator.

SOURCE OF CHECK CULTIVARS AND SCIENTISTS WITH EXPERTISE

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HELPFUL INFORMATION: An optimal, qualitative (dead or alive) reaction occurs when plants are growing rapidly. Controlled greenhouse conditions, optimum pH and fertility, and, direct sunlight are ideal for separation of tolerant and non-tolerant reactions. Under sub-optimal conditions non-tolerant seedlings, although permanently injured, may survive. Plant stress will delay symptoms and or will confound reaction (e.g., take precautions so that damping-off is avoided). It is helpful to plant 1 row of a non-tolerant entry with 9 rows of the test variety per flat so that herbicide exposure and uniformity can be monitored. *Note:* Any of the labeled Roundup formulations with our without surfactant may be used, however, at the present date, all formulations federally labeled for use on Roundup Ready® alfalfa contain surfactant. Scoring of the reaction phenotype (growth vs. collapse) is not adversely affected by use of surfactant-containing formulations. Surfactants aid in proper leaf wetting.

ALTERNATIVE METHODS: The grow-out method (described above) gives an easy-to-score, clear-cut reaction. Germination assay formats (e.g., blotter/towel/agar plate tests) result in subjectively-scored tolerance phenotypes, and while potentially useful, these assays are not recommended at this time for critically measuring trait purity in Roundup Ready® varieties.

TERMS OF USE: Roundup® and Roundup Ready® are trademarks of Monsanto Company. Any non-labeled use of Roundup® herbicides for research purposes is the sole responsibility and liability of the user. The application rate in this procedure exceeds the approved product field label rate and all treated plants and tissues should be destroyed at the conclusion of the evaluation.

**This standard test should only be used to measure trait purity of Roundup Ready varieties; this test should not be used to evaluate adventitious presence (AP) or document herbicide crop safety claims per se.*