

Highlights:

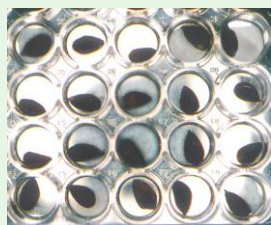
- Recognizes AAD-12, Cry1Ac, Cry1F, CP4 EPSPS, & Vip3A traits
- Results in 10 minutes or less
- Strips in convenient comb format

Contents of Kit:

- 48 QuickStix Combo Strips assembled as 6 combs of 8 strips packaged in a foil bag
- Buffer Packet (makes 1L)

Items Not Provided:

- Seed crusher
- Repeating pipetter or other means of dispensing 0.5 mL per well (delinted) and/or 0.25 and 0.5 mL per well (fuzzy)
- Pin plate or other means of stirring extract (fuzzy)
- Distilled or deionized water for preparing Buffer



Load seeds in wells, **crush** twice



Add Extraction Buffer

Catalog Number AS 175 STC

Part # 12732

Intended Use

The EnviroLogix QuickStix Combo Comb Kit for Multi-Trait Testing is designed to detect the presence AAD-12, Cry1Ac, Cry1F, CP4 EPSPS, and Vip3A proteins at levels typically expressed in WideStrike® 3, Genuity® Roundup Ready® Flex, Enlist™ cotton seed tissue. The combo strips will recognize AAD-12, Cry1Ac, Cry1F, CP4 EPSPS and Vip3A proteins in separate regions of the same strip.

How the Test Works

Cotton plants and seeds that have been genetically modified with stacked genes express transgenic proteins in their seed. To detect these proteins with the EnviroLogix QuickStix Combo Strip, the sample must be extracted and the proteins solubilized in the buffer provided.

Each Combo Strip has an absorbent pad at each end. The protective tape with the arrow indicates which end of the strip to insert into the sample. The sample travels up the membrane strip and is absorbed into the larger pad at the top of the strip. The portion of the strip between the protective tape and the absorbent pad at the top of the strip is used to view the reactions as described under “Interpreting the Results”.

Sample Preparation

Before testing, prepare Buffer: Add the contents of the Buffer Packet to 1 liter of distilled or de-ionized water (room temperature) and stir to dissolve. Store in closed container and refrigerate when not in use; warm to room temperature prior to assay.

A. Delinted Seed:

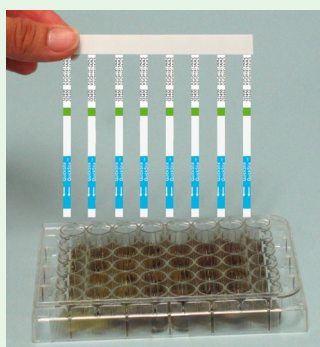
- A1. Load individual delinted cotton seeds into each of the 48 wells.
- A2. Crush seeds with hydraulic press using 4 to 5 tons of pressure. Turn the plate around ninety degrees and crush the seeds again. **It is critical that the seeds are crushed twice using 4 to 5 tons of pressure to ensure test line development.**
- A3. The crushed state of the seed is visible through the bottom of the microplate or by gently lifting the crusher. Gently shake the crusher while lifting to dislodge any seed. Use extreme care, do not cross-contaminate the wells! **Clean crusher prongs prior to using on the next plate.**
- A4. Add 0.5 mL of the prepared, **room temperature Buffer** to each well and cover.
- A5. Mix on orbital shaker or equivalent for **10 minutes**. Remove and discard the plate cover. **Ensure seed extract has a yellow to cloudy gray color before adding the combs.**
- A6. Use crushed seed samples the same day they are prepared.

To improve extraction efficiency:

- Use 80 to 90°F buffer to extract the seeds.
- Longer soak times can increase the strength of the Test Line (better extraction).



Remove QuickStix Combs



Insert Combs into plate

Any clearly discernable pink Test Line is considered positive

- If seed material gets stuck to the plate bottom, use the QuickStix to gently mix the extract as soon as you insert it into the well.
- The extract takes on a yellow to brown opaque color when the seeds are crushed and mixed properly. If the extract is clear, the seed coat may be empty or the sample may not be well mixed. The seeds should contain an adequate amount of mature endosperm and embryonic tissues, not empty seed coats.

B. Fuzzy Seed:

- B1. Add 0.25 mL of Extraction Buffer to each well.
- B2. Remove any extra lint on the fuzzy cotton seeds and load one seed into each of the 48 wells.
- B3. Crush seeds twice with hydraulic press (4-5 ton) or equivalent.
- B4. Gently shake the crusher while lifting to dislodge any seed. Use extreme care, do not cross-contaminate the wells! **Clean crusher prongs prior to using on the next plate.**
- B5. Add 0.5 mL of Extraction Buffer to each well and cover.
- B6. Mix covered plate on orbital shaker (~600 rpm) or equivalent for 10 minutes. Remove and discard the plate cover.
- B7. Use pin plate or equivalent to vigorously break up seeds and mix with extract until all wells exhibit cloudy extract. Use extreme care not to cross-contaminate the wells. **Clean pin plate prior to using on next plate.**
- B8. Use crushed seed samples the same day they are prepared.

How to Run the QuickStix Comb Test

1. Allow refrigerated foil bag to come to room temperature before opening. Remove a QuickStix Comb from the bag and reseal the bag. A blank space is provided to label each comb if desired. Place the comb of strips into the plate wells, being sure to insert the end indicated by the arrows on the protective tape.
2. After inserting the comb into the extracts, liquid will travel up the membrane strips toward the absorbent pads at the top of the strips. Soon after complete wetting of the membrane, a line will appear on the membrane approximately 1/8 inch below the top absorbent pad. This is the Control Line.
3. The results should develop within 6 to 10 minutes. Allow the strips to develop for a full 10 minutes before making final negative assay interpretations. Strongly positive samples may show results much sooner. Remove the QuickStix Comb from the wells to read. To retain the sticks, cut off and discard the bottom section of each strip covered by the arrow tape. Results of the wet strips should be recorded.

Interpreting the Results

Development of the Control Line within 10 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded and the sample re-tested using another strip.

One Line – If the extract is from a negative sample, containing none of the target proteins, the strip will only show the Control Line, about 1/8 inch below the top pad.

Two to Six Lines – If the extract is from a sample containing AAD-12, Cry1Ac, Cry1F, CP4 EPSPS and/or Vip3A proteins, the appropriate test line(s) will appear. Please refer to the interpretation guide below.



	WideStrike 3, Genuity Roundup Ready Flex, Enlist	WideStrike 3, Genuity Roundup Ready Flex	WideStrike 3	WideStrike	Genuity Roundup Ready Flex or Roundup Ready	Conventional
Control Line	✓	✓	✓	✓	✓	✓
AAD-12 Test Line	✓					
Cry1Ac Test Line	✓	✓	✓	✓		
Cry1F Test Line	✓	✓	✓	✓		
CP4 Test Line	✓	✓			✓	
Vip3A Test Line	✓	✓	✓			

✓ indicates positive Line development for given trait
 □ indicates no Test Line development for given trait

Please note that the order of the lines from the top of the strip containing the absorbent pad to the bottom of the strip (arrow tape) is: AAD-12, Cry1Ac, Cry1F, CP4 EPSPS, and Vip3A.

Kit Storage

This Kit can be stored at room temperature, or refrigerated for a longer shelf life. Please note the shelf life on the kit label for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the foil bag until ready to use the test strips.

Precautions and Limitations

- This kit is designed for screening for presence or absence only and is not meant to be quantitative.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized to be used with the protocol and with the buffer provided in the kit. Deviation from this protocol may invalidate the results of the test.
- A strong positive result may safely be interpreted in as little as 6 minutes after sample addition. It is not safe, however, to conclude that a sample is negative before a full 10 minutes has elapsed.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.



**For Technical Support
Contact Us At:**

EnviroLogix
500 Riverside Industrial
Parkway
Portland, ME 04103-1486
USA

Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:
info@envirologix.com

website:
www.envirologix.com



LIMITED WARRANTY

EnviroLogix Inc. (“EnviroLogix”) warrants the products sold hereunder (“the Products”) against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. If the Products do not conform to this Limited Warranty and the customer notifies EnviroLogix in writing of such defects during the warranty period, including an offer by the customer to return the Products to EnviroLogix for evaluation, EnviroLogix will repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

ENVIROLOGIX MAKES NO OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EnviroLogix products appearing in EnviroLogix published catalogues and product literature are EnviroLogix’ sole representations concerning the Products and warranty. No other statements or representations, written or oral, by EnviroLogix’ employees, agents or representatives, except written statements signed by a duly authorized officer of EnviroLogix Inc., are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.

EnviroLogix does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by EnviroLogix, or against damages resulting from such non-EnviroLogix made products or components. EnviroLogix passes on to customer the warranty it received (if any) from the maker thereof of such non-EnviroLogix made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by EnviroLogix.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of EnviroLogix shall be to repair or replace the defective Products in the manner and for the period provided above. EnviroLogix shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall EnviroLogix be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of EnviroLogix with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

WideStrike is a trademark of Dow AgroSciences LLC
Genuity and Roundup Ready are registered trademarks of Monsanto Technology LLC
Enlist is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow
EnviroLogix, the EnviroLogix logo, and QuickStix are trademarks of EnviroLogix Inc.

© EnviroLogix 2019