



Catalog Number AS 049 GP 25

Intended Use

The EnviroLogix QuickStix Kit for *Botrytis* in Wine Grape Juice screens for the presence of *Botrytis cinerea*. The test can provide qualitative or semi-quantitative results. A qualitative result indicates the presence or absence of *Botrytis* infection, with a sensitivity of 5 BCAGU, which equates to 0.625% infection by incidence in grape berries. See “Limit of Detection,” page 2.

A semi-quantitative result is provided when used in conjunction with the QuickStix Reader™. The Reader measures the strength of the test signal and correlates that with a percentage of infection, by incidence or weight, as determined by the Dewey I-W Standard (see page 2). The QuickStix Reader is not included in this kit but is available through EnviroLogix (Cat #ACC-031).

Highlights:

- Qualitative field test with results in 10 minutes or less
- Semi-quantitative results using the QuickStix Reader
- Highly sensitive test with objective infection measurement scale (for use with QuickStix Reader)
- Developed in conjunction with leading university programs and experts in viticulture diseases
- Replaces time-consuming, subjective and costly hand-sorting evaluation

Contents of Kit:

- 25 QuickStix Strips packed in a moisture-resistant canister
- 25 reaction vials
- 25 transfer pipettes
- EB8 Extraction Buffer

Items Not Provided:

- QuickStix Reader™ (Cat# ACC 031, optional)
- Pipette & tips
- Sieve/filter
- Container for mixing (e.g. Falcon vial)

How the Test Works

This test is for use on juice from wine grapes that is suspected of being infected with *Botrytis*. To detect *Botrytis* antigens in the juice of wine grapes with the EnviroLogix QuickStix strip, samples must be diluted with the buffer provided according to the Sample Preparation directions below.

Each QuickStix strip has an absorbent pad at both ends. The protective tape with the arrow indicates which end of the strip to insert into the extraction tube. 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

1. To prepare grape juice for testing, select a representative sample of grapes and squash or macerate. To avoid pulp in sample to be tested, sieve mashed grapes through a plastic, nylon or other non-absorbent coarse filter (not provided).
2. Dilute grape juice 1:40 into EB8 Buffer. It is recommended that 200 µL of sample be diluted into 7.80 mL of buffer to make the proper dilution. Be sure to use a new pipette tip for every sample to avoid potential cross-contamination. Please see "Developing a Standard" in the Notes section for details regarding different dilution ratios to further refine results.
3. Mix by shaking or inverting, using an appropriate water-tight mixing vessel.
4. Use the transfer pipette provided to fill the reaction vial to the top of the tapered region (approximately 500 µL).

How to Run the QuickStix Strip Test

1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place the strip into the filled reaction vial. The sample will travel up the strip. Reaction vials will stand on their own or may be inserted into the cardboard racks provided.
3. Allow the strip to develop for a full 10 minutes before making final assay interpretations. Positive sample results may appear much more quickly.



Any pink test line is considered positive

The Dewey I-W Standard:

The Dewey I-W Standard was developed by Frances M. Dewey, PhD, through her work with the UC Davis Viticulture program. Dr. Dewey, recognized for her work in Botrytis, developed this standard in response to industry requests for a more objective measurement of Botrytis infection in grapes or grape juice. For reference to articles and data produced by Dr. Dewey, please visit our website.

- Measures Botrytis levels in wine grape juice on the basis of incidence or weight.
- Incidence (or berry/berry) is derived from a 20 % standard, which combines 20 half-turgid, Botrytis-infected Chardonnay berries with 80 uninfected berries. This juice mix is then diluted further into juice from other uninfected berries to achieve a measurement range.
- Weight (or weight/weight) is obtained by multiplying the incidence level by 0.333, as a half-turgid, Botrytis-infected wine grape berry weighs, on average, one-third that of an uninfected berry.
- The Dewey I-W Standard (curve) is preprogrammed into the EnviroLogix QuickStix Reader for automatic quantification of the results.

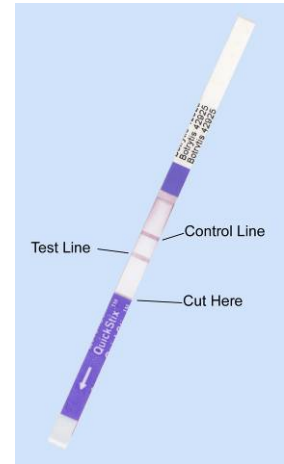
4. Read and interpret the results as close as possible to the 10 minute mark, while the strip is still in the reaction vial. If using the EnviroLogix QuickStix Reader, it is important to remove the bottom pad (leaving the backing material) before placing the strip into the strip holder. Reading the strip should be done immediately upon removal from the reaction vial, while the strip is still wet. Removing the bottom pad stops the reaction at the appropriate time, while leaving the backing material ensures the QuickStix fits properly into the holder.
5. To retain the strip, cut off and discard the bottom section of the strip covered by the arrow tape.

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.

If the sample extract contains *Botrytis*, a second line (Test Line) will develop on the membrane strip between the Control Line and the protective arrow tape. The results should be interpreted as positive for *Botrytis*.

If no Test Line is observed after 10 minutes, the results should be interpreted as negative.



Limit Of Detection (LOD)

The LOD is expressed in “*Botrytis cinerea* Antigen Units,” which is expressed as BCAGU. One (1) BCAGU is equal to one (1) µg of *Botrytis cinerea* freeze-dried mycelial extract per mL of sample extract).

When applying the 1:40 sample dilution protocol described herein, the LOD in the diluted sample is 5 BCAGU. Therefore, prior to dilution the sample must contain ≥200 BCAGU.

Greater test sensitivity may be achieved for measuring the level of *Botrytis* in wine grapes and juice from other markets using their standards. Please see "Developing a Standard" in the Notes section for details regarding different dilution ratios to refine results.

Interpreting Semi-Quantitative Results with the QuickStix Reader

1. Follow Reader guide for placing strips into the reading device.
2. If the strip is not barcoded, the initial bar code scan will “fail.” Manually select the appropriate measurement test protocol from the reader display (measuring contamination by incidence or weight as desired). Enter the strip lot number at the prompt.
3. The table below illustrates the range of possible results, with each signal intensity code corresponding to a specific % Incidence and % Weight for infected grape juice.

Signal Intensity	% Infection, by Incidence	% Infection, by Weight
<10	< 1.25	< 0.42
10 - 23.5	≥ 1.25 to < 2.50	≥ 0.42 to < 0.83
23.5 - 31.0	≥ 2.50 to < 5.00	≥ 0.83 to < 1.67
31.0 - 42.5	≥ 5.00 to < 10.00	≥ 1.67 to < 3.33
>42.5	≥ 10.00	≥ 3.33



Place QuickStix Strip in holder,
insert into QuickStix Reader

4. Consult the QuickStix Reader manual for more detailed instructions on use of the QuickStix Reader.

Note: If the sample is giving results of an SI greater than 42.5, it is possible to make an additional dilution of the sample into the EB8 buffer and re-run it in the assay. Once a post extraction dilution (PED) allowing the sample to fall within the assay's quantitative range (greater than or equal to 10 and less than 42.5) is reached, the resulting quantification range will need to be adjusted by the PED employed. Please call EnviroLogix Technical Service if further explanation or clarification is needed. Refer to the Notes section for details on "Developing a Standard" with different dilution ratios for different situations.

Kit Storage

This QuickStix Kit should be stored refrigerated. Note the shelf life on the kit box. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the strips.

Cross Reactivity

The antibody used to produce this kit was found to be reactive with:

- *Botrytis cinerea* (various isolates)
- *B. byssoidea*
- *B. allii* (various isolates)
- *B. fabae*
- *B. squamosa*
- *B. stokesii* (tulipae; various isolates)
- *B. streptothrix*
- *B. tulipae*
- *B. aclada*
- *B. elliptica*
- *Monilinia fructicola*
- *M. laxa*
- *M. lambertella*
- *Sclerotinia* spp.

The antibody used to produce this kit was found to be not reactive with:

- *Alternaria infectoria*
- *Alternaria alternata*
- *Aspergillus niger*
- *Aureobasidium pullulans*
- *Cladosporium macrocarpum*
- *Cladosporium herbarum*
- *Cladosporium paeoniae*
- *Coniella fragaria*
- *M. hiemalis*
- *Rhizopus* spp.
- *Stemphyllium* spp.
- *Trichoderma harzianum*
- *T. viride*
- *Ulocladium* spp.



Precautions and Notes

- The QuickStix Strips are designed for screening the presence or absence of *Botrytis* and are not meant to be quantitative. Semi-quantitative results can be obtained using the strips in conjunction with a QuickStix Reader.
- Important Note: The kit will detect its target pathogen regardless of the pathogen's viability. It should not be used to determine the efficacy of treatment efforts, because although the pathogen may be rendered non-viable, the glycoprotein is still present and will cause a positive result.
- As with all tests, it is recommended that results be confirmed by an alternate method.
- The assay has been optimized to be used with the protocol provided in the kit. Deviation from this protocol may invalidate the results of the test. Compositing or pooling of samples is not recommended and may result in false negative results.
- A negative result does not preclude the presence of *Botrytis* infection in other areas or at other times.
- A strong positive result may safely be interpreted in as little as 5 minutes after sample addition. It is not safe, however, to conclude that a sample is negative



before a full 10 minutes has elapsed. A weakly positive sample may require the full 10 minutes for a distinct Test Line to appear.

- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle. A small portable cooler is recommended for field testing applications to protect the kit from extreme temperatures.

- **Developing a Standard:**

The test protocol developed and validated by EnviroLogix in conjunction with Molly Dewey Ph.D. is based on a 1:40 dilution ratio, determined to be a dilution that eliminated most differences among grape varieties and conditions. It is the basis for the Dewey I-W Standard that is referenced on Page 2, and is coded into the QuickStix Reader by default.

Because this Standard and the 1:40 dilution ratio may not serve every customer's needs, developing an individualized set of standards will return results that are more tailored to the individual user's needs. Use the following process:

- Identify a standard of interest by collecting a juice sample that represents the threshold for concern. Make multiple dilution of that **threshold sample** (1:5, 1:10, 1:20 etc.) and test each with the QuickStix, reading the results with the QuickStix Reader.
- Select the dilution level that gives an SI close to the center of the dose response curve (SI = approx. 25-30). This will determine the **optimum dilution level**. If unable to get the SI in the middle of the curve, use the lowest level (1:5) to limit the separation between levels below the standard of interest.
- Test clean berries (of the varieties to be tested) at the optimum dilution level to make sure they give a negative result (SI less than 2, visually clean).
- Make the test semi-quantitative by preparing standards from the original threshold sample. For example, serially dilute the threshold sample into negative grape juice (keeping the matrix consistent), dilute using the optimum dilution level, and test with the QuickStix. When running an unknown, this will serve as a reference to where the sample falls; for example, the sample is giving an SI equivalent to 1/2 the threshold.
- The QuickStix Reader can be re-programmed with an individual standard protocol (or many), rather than or along with the default protocol, to quickly and easily quantify *Botrytis*. The Reader curve choices can be labeled to easily select the protocol used, and return highly relevant results relative to the threshold level of interest.



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