TotalTarget™ Kit for Sprout Damage in Wheat

Catalog Number AT-SD-W *Part #13780*

Matrices and Detection Ranges:

Matrix Group ID	Protocol	Results reported in the range of:
MG1 –Freshly Ground Soft Red Wheat	Standard Protocol	175-350 seconds
MG2 –Freshly Ground Soft White Wheat	Standard Protocol	175-400 seconds
MG3 – Freshly Ground Soft Wheat (EU)	Standard Protocol	175-400 seconds
MG4 – Freshly Ground Wheat (Brazil)	Standard Protocol	175-400 seconds
MG5 – Freshly Ground Hard Red Wheat	Standard Protocol	175-450 seconds

Matrices:

Note: Scanning the Multi-Matrix Barcode Card once per kit lot is required. The QuickScan software will prompt users to select a Matrix Group (MG) before proceeding to the result screen.		
▪ Freshly Ground Soft White Wheat, and Soft Red Wheat	DB6 Buffer Extraction, Substrate Tablets	PAGE 3

Intended Use

The TotalTarget Kit for Sprout Damage in Wheat is designed for the quantitative detection of alpha-amylase protein activity in bulk grain samples of North American soft red (MG1) and white winter wheat (MG2), European soft wheat (MG3) and Brazilian wheat (MG4). This single-strip assay provides results that can be automatically and approximately correlated to a Hagberg falling number value using the QuickScan lateral flow reader platform. The assay detects alpha-amylase activity corresponding to a Hagberg falling number of approximately 175 to 350 seconds with MG1/MG2, and approximately175 to 400 seconds with MG3/MG4.

Contents of Kit:

- 50 Sprout Damage Strips packed in a moisture-resistant canister
- 50 Sprout Damage Substrate Tablets packed in a moisture-resistant canister
- 50 reaction tubes
- 100 pipette tips (100 - 1,000 µL)
- 17 mL DB6 Buffer (lot specific)
- 1 Multi-Matrix Barcode Card - kit lot specific

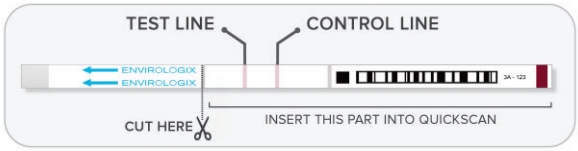
Important Notes:

- Before testing, the enclosed Multi-Matrix Barcode Card (MMBC) must be scanned just once for each kit lot to upload information to the QuickScan
- QuickScan Software Version 5.13.0 or later is required

How the Test Works

The TotalTarget Kit is a "competitive" type lateral flow strip test. In the test, wheat is prepared and tested using a strip coated with antibodies reactive against a target found in the tablets. Amylase presence in the sample digests starch from the tablet, freeing up a molecule which will also bind to the gold conjugate. Normally the gold conjugate will bind to the test line, but with the "competing" molecule from the starch, it will not attach to the test line. The effect from the competing molecule is that a low amylase activity sample will have a strong test line signal, where a high amylase activity sample will have a comparatively weak test line.

A composite sample is collected, ground, and extracted to solubilize any alpha-amylase present. The extract is further diluted into a dilution buffer along with a substrate tablet that helps to drive the test before being run on the test strip. Each strip has an absorbent pad at each end. The sample extract travels up the test strip and is absorbed into the larger pad at the top of the strip. At the end of the reaction time, the strip is cut at the top of the arrow tape, the bottom pads are removed, and the strip is inserted into the QuickScan reader to obtain a quantitative result, which is then approximately correlated to a Hagberg falling number value.



Items Not Provided:

- QuickScan System*
- Incubator (base + block)*
- Mill grinder or equivalent
- 20-mesh screen (available through Seedburo or other vendor)
- Vortex-Genie 2 or equivalent with flat 3" or greater head
- Digital scale for weighing samples
- Extraction cups with lids*
- Graduated cylinder*
- Orbital/rotary shaker
- Centrifuge capable of 2000 x *g (not rpm)*, and accessories*
- Pipette to deliver 100-300 µL*
- Approved coffee filters*
- Timer
- Scissors
- Distilled, deionized, or bottled water

**Available as accessories*

***Available Accessories:**

Item	*Catalog No.*	*Part #*
QuickScan II™ System	ACC-351	13065
5 oz Sample cups/lids *Case of 500; for extracting samples up to 30g*	20-0047	10167
10 oz Sample cups/lids *Case of 100; for extracting samples >30g*	20-0129	12383
Graduated cylinder (100 mL)	ACC 068	11207
Reusable 8" Tweezer	ACC 089	14000
Accumax pipette 100-1000 µL	ACC 1303	11964
Centrifugation Set: *Disposables for 50 tests*	ACC 010	11214
Microcentrifuge	ACC 064 E	11204
DB6 Buffer	KR-268-7	12452
Incubator	ACC BSH 301	12458
Coffee Filters (100 pk)	ACC 083	11434

Sample Preparation

- Collect a composite sample according to your own sampling plan or USDA/FGIS (formerly GIPSA) guidelines, a 250g or greater sample is recommended. Consult USDA reference documents to help design a plan that fits your needs. Contact Technical Support for more information.
- Ensure all reagents, including samples, strips, buffer, and substrate tablets, are at room temperature (68-75 ºF or 20-24 ºC) and ready for use before starting the assay.
- Grind samples to provide a consistency such that 95% passes through a 20-mesh sieve.
- Mix ground material thoroughly before sub-sampling, to minimize variability.
- Weigh 20 to 50g samples into containers that will allow enough head room for the liquid to move forcefully when shaken vigorously.

Sample Extraction

1. **Extract** sample in water. Use distilled, deionized, or bottled water. Weigh out ground, homogenized sample and add to a sample cup, using a graduated cylinder add water; for every 1 g of sample, use 5 mL of water (i.e. a recommended 20 g ground wheat sample would be mixed with 100 mL of water).

20 - 30 g samples	*31 – 50 g samples*
• Use a 5 oz sample cup (part number 10167)	• Use a 10 oz sample cup (part number 12383)
Wet sample immediately, by vigorously shaking for 5 seconds by hand until sample is fully wet. If needed, shake the sample against the palm of your other hand or a hard surface to loosen up any dry sample areas. **Immediately** proceed to next shaking step.	

2. **Shake** sample, mechanically or by hand.

Shaker Table: mix at highest speed (≥ 300 rpm) for 30 seconds	***By Hand:*** shake vigorously for 30 seconds

3. **Clarify** sample using one of the two methods below (A or B):

   A) Centrifugation: Using a clean transfer pipette immediately fill a microcentrifuge tube with extract and centrifuge it for 30 seconds at 2000 x *g* (not RPM). Consult centrifuge manual for *g* force calculation and follow manufacturer's instructions for operation and balancing.

   B) Filtration: Add an approved coffee filter to a clean vessel, with enough space for the clarified extract to collect, and pour the extract into the filter and allow it to sit for no longer than 2 minutes (sufficient extract will be available well before this point). Pull back an edge of the filter to gain access to the clarified extract.

TIPS!

Get Complete Extraction
- Fully wet samples before the next shaking step
- Avoid delay between water addition and shaking
- Assure liquid is moving forcefully though the sample while shaking

For Best Performance
- Pipette up and down to mix
- Read strips promptly after run time

Avoid Contamination
- Use a new Reaction Tube per test
- Keep DB6 capped, when possible
- Use new pipette tips for each step and for each new sample

How to Run the TotalTarget Sprout Damage Test

- Review the Sample Preparation instructions above for grinding consistency and notes.
- Ensure the ambient temperature of the air is between 68-75°F (20-24°C). If the room temperature is outside of this range the accuracy of the results may be impacted.
- Turn on the incubator and set to ***70 °C** for up to 20 minutes before testing. Ensure that the temperature display has stabilized and indicates "OK" before starting the assay. All reagents should be at room temperature for use.

  *Periodically check the temperature of the heat block with a calibrated external thermometer to ensure it is between 68-72°C. If the heat block is outside of this range the accuracy of the results may be impacted.

  1. Using clean forceps, tweezers, or gloved hands, place one Sprout Damage Substrate Tablet into a Reaction Tube.
  2. Transfer 300 µL of the clarified extract to the Reaction Tube. If you clarified the extract using centrifugation avoid drawing up floating top layer (use the clear sample just below this layer when present).
  3. Vortex the reaction tube at max speed for 15 seconds.
  4. Immediately place the reaction tube in the incubator set for 70 ºC for 90 seconds (use the center location of the incubator when possible).
  5. **IMMEDIATELY** at 90 seconds, add 300 µL DB6 (lot specific) to the reaction tube (discard tip) while still in the incubator, remove the tube, and vortex at max speed for 5 seconds. Place the reaction tube in a rack, **do not put back into the incubator.**
  6. Allow the reaction tube to rest for 30 seconds at room temperature.
  7. Add test strip to tube, arrows down, allow strip to develop for 3 minutes (run time).
  8. Immediately cut strips at the top of the arrow tape (discard bottom pads).
  9. Insert strip into the QuickScan Reader and click "Read Test".
  10. The QuickScan will return a result that approximately corresponds to a calculated Hagberg falling number value (ex: 325 seconds).

Use of the QuickScan System

Detailed instructions for use of the QuickScan System are supplied with each unit and can also be found at www.envirologix.com/support/quickscan/. The lot-specific Multi-Matrix Barcode Card (MMBC) must be scanned into the system prior to testing. In summary, a strip is inserted into the reader and the strips are read by touching or clicking on the "Read Test" area of the screen. The "Select Matrix Groups" screen will appear if more than one barcode was scanned into the system from the MMBC. Select the group that displays the matrix run. Results are then recorded in an electronic worksheet, allowing each user to report and track data easily.

Kit Storage

TotalTarget Sprout Damage strips can be stored in their tightly capped canister at room temperature or refrigerated for a longer shelf life. Note the shelf life on the kit box for each storage temperature. **NOTE: The DB6 buffer must be stored refrigerated.** 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 test strips.

Notes

- This test is designed to be run alongside the TotalTox DON (deoxynivalenol) assay (catalog number AQ-304-BG). Recommended instructions for a combined workflow can be found on page 8.
- This product is currently not applicable for use in testing any other crops beyond those specified in this Product Insert.
- This kit is designed to give a result using the QuickScan System and is not intended to be visually interpreted.
- Avoid contaminating the kit's tablets when being stored or handled to ensure that no additional alpha-amylase affects test results.
- Avoid contaminating the DB6 buffer provided in the kit. Once a pipette tip has been in contact with the sample, it should not be returned to the DB6 bottle, and a new tip should be used.
- 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.
- References to room-temperature within this assay refers to the temperature range of 68-75^{o}F or 20-24^{o}C.
- With all tests, it is recommended that results be confirmed by an alternate method if necessary.
- Pipettes lose calibration accuracy over time. Calibrate or replace pipettes at least annually.
- The recommended incubator (Cat# ACC BSH 301) comes pre-calibrated but may experience drift over time which can be confirmed with a separate, calibrated thermometer. Periodic recalibration is recommended to mitigate temperature drift, instructions in the user manual may be followed for recalibration.
- The results generated through the proper use of this diagnostic tool reflect the condition of the working sample directly tested. Extrapolation as to the condition of the originating lot, from which the working sample was derived, should be based on sound sampling procedures and statistical calculations which address random sampling effects, non-random seed lot sampling effects and assay system uncertainty. A negative result obtained when properly testing the working sample does not necessarily mean the originating lot is entirely negative for the analyte or protein in question.
- DO NOT leave in direct sunlight or in vehicle. Protect all components from hot or cold extreme temperatures when not in use.
- Samples should be ground within 24 hours prior to testing and remain stable for up to 24 hours when stored at room temperature.



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Safety data sheet

SECTION 1. Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier
Trade name: **DB 6 Dilution Buffer**
Part number 11151 (KR-268)

1.2 Relevant identified uses of the substance or mixture and uses advised against application of the substance / the preparation : Laboratory chemicals; kit component. Not to be used for purposes other than those specified in product literature.

1.3 Details of the supplier of the safety data sheet
Manufacturer/Supplier: EnviroLogix Inc., 500 Riverside Industrial Pkwy, Portland ME 04103, USA
Phone: (207) 797-0300

1.4 Emergency telephone number: (207) 797-0300 Technical Service

SECTION 2. Hazards identification.

2.1 Classification of the substance or mixture

Classification according to 29CFR 1910.1200: Not Classified

2.2 Label elements
Labeling according to 29CFR 1910.1200

Pictogram: None
Signal word: None
Hazard Statements: None

2.3 Other Statements: None

SECTION 3. Composition/information on ingredients.

3.2 Mixture

Chemical name	CAS No	EC No	Classification According to 29CFR 1910.1200	Amount (%)
Sodium Tetraborate Decahydrate	1303-96-4	215-540-4	H360 Rep 1B	< 3 %

SECTION 4. First aid measures.

4.1 Description of first aid measures

After inhalation : In case of inhalation. Remove to fresh air. If not breathing give artificial respiration. Get medical attention immediately.

After skin contact : In case of skin contact. Remove contaminated clothing and shoes immediately. Wash affected area with mild soap or detergent for at least 10 minutes or until no evidence of chemical remains.

After eye contact : In case of eye contact, immediately flush eyes with plenty of water for at least 15 minutes. Lifting eyelids occasionally, until no evidence of chemical remains. Get medical attention immediately.

After swallowing : In case of ingestion. DO NOT Induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call a physician immediately.

4.2 Most important symptoms and effects, both acute and delayed: None

4.3 Indication of any immediate medical attention and special treatment needed: None

SECTION 5. Firefighting measures.

5.1 Extinguishing media: CO_2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.

5.2 Special hazards arising from the substance or mixture: None

5.3 Advice for firefighters: Wear protective gear appropriate for fire conditions including respiratory protective gear.

SECTION 6. Accidental release measures.

6.1 Personal precautions, protective equipment and emergency procedures: In the case of spilled mixture wear gloves to prevent skin contact. In the case of a large spill, additional protection is recommended.

6.2 Environmental precautions: Do not discharge mixture to sewer system or waterways.

6.3 Methods and material for containment and cleanup: Absorb in paper towel or suitable absorbent for larger spills and discard in appropriate waste. Clean with water afterwards.

6.4 References to other sections: For safe handling refer to Section 7. For information on PPE refer to Section 8. For disposal refer to Section 13

SECTION 7. Handling and storage.

7.1 Precautions for safe handling: Practice good chemical hygiene when handling. Avoid contact with eyes, skin, and clothing.

7.2 Conditions for safe storage, including any incompatibilities: Store in tightly closed, non-metal container, in a corrosive compatible area. Prevent direct sunlight and heat. Store in well aired storage rooms.

7.3 Specific end use(s): Apart from the uses mentioned in section 1.2, no other specific uses are stipulated

SECTION 8. Exposure controls/personal protection.

8.1 Exposure limits:
Components with limit values that require monitoring at the workplace:

	EH40/2005	OSHA
Sodium Tetraborate Decahydrate	8 Hr TWA = 5mg/m^3	8 Hr TWA = 10 mg/m^3

8.2 Exposure Controls:

8.2.1 Engineering controls: Facilities using this mixture should be equipped with an eyewash and safety shower. Use general or local exhaust ventilation to keep airborne concentrations below permissible exposure limits.

8.2.2 General protective and hygienic measures: The usual precautionary measures should be adhered to when handling chemicals.

Eye Protection: Safety glasses with side shields, goggles. Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166 (EU). Eye and face protection regulations are described by OSHA (US) in 29CFR1910.133. Do not wear contact lenses when working with chemicals

Hand Protection: Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands. The selected protective gloves have to satisfy the specifications of EU Directive 89/686/EEC and the standard EN 374 derived from it.

Breathing Equipment: Appropriate respiratory protection should be determined according to local conditions using risk analysis protocols. An approved disposable air purifying particulate respirator may be used as a backup to engineering controls. Always use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

8.2.3 Environmental exposure controls: Contain spills, do not allow into environment

SECTION 9. Physical and chemical properties.

9.1 Information on basic physical and chemical properties:

a) Appearance: Clear liquid, colorless to slight yellow.
b) Odor: None
c) Odor Threshold: No Data Available
d) pH: 8.6
e) Melting point/freezing point: No Data Available
f) Boiling point/Boiling range: No Data Available.
g) Flash point: Not applicable.
h) Evaporation rate: No Data Available
i) Flammability (solid, gaseous): No Data Available
j) Upper/lower flammability or explosive limits: No Data Available
k) Vapor pressure: No Data Available
l) Vapor density No Data Available
m) Relative density: No Data Available
n) Solubility(ies): Fully miscible, water.
o) Partition Coefficient: n-Octanol/water: No Data Available
p) Auto-ignition temperature: No Data Available
q) Decomposition temperature: No Data Available
r) Viscosity: No Data Available
s) Explosive properties: No Data Available.
t) Oxidizing properties: No Data Available

9.2 Other information: No further relevant information available.

SECTION 10. Stability and reactivity.

10.1 Reactivity: No data available

10.2 Chemical Stability: Stable under normal temperatures and pressures.

10.3 Possibility of hazardous reactions: Under normal conditions of storage and use, hazardous reactions will not occur.

10 4 Conditions to avoid: No specific data

10.5 Incompatible materials: No Data Available.

10.6 Hazardous decomposition products: Under normal conditions of storage and use, hazardous decompositions products should not be produced.

SECTION 11. Toxicological information.

Information on Toxicological Effects

Acute effects (toxicity tests): No Data Available

Sensitization: No sensitizing effects known

CMR (carcinogenicity, mutagenicity and toxicity for reproduction) effects: No CMR effects.

Additional toxicological information: No Additional Information

SECTION 12. Ecological information.

12.1 Toxicity: No Data Available

12.2 Persistence and degradability : No Data Available

12.3 Bio accumulative potential: No Data Available

12.4 Mobility in soil : No Data Available

12.5 Results of PBT and vPvB assessment: Not available as a chemical safety assessment, not required/not conducted.

12.6 Other adverse effects: No Data Available

SECTION 13. Disposal considerations.

Waste treatment methods: Contact a licensed professional waste disposal service to dispose of this material. Disposal of surplus or waste solutions must be in accordance with applicable local, state, and national laws and regulations.

SECTION 14. Transport information.

14.1 UN-Number DOT, ADR, ADN, IMDG, IATA :	Not Hazardous for Transport
14.2 UN proper shipping name DOT, ADR, ADN, IMDG, IATA :	Not Hazardous for Transport
14.3 Transport hazard class(es) DOT, ADR, ADN, IMDG, IATA):	Not Hazardous for Transport
14.4 Packing group (DOT, ADR, IMDG, IATA):	Not Hazardous for Transport
14.5 Environmental hazards	No environmental hazard.
14.6 Special precautions for user :	None
14.7 Transport in bulk according to Annex II of MARPOL73/78 and the IBC code:	No information available.

SECTION 15. Regulatory information.

15.1 Safety, health, and environmental Regulations	Not a hazardous material
US Federal Regulations	
OSHA	Not listed
SARA 313	
US State Regulations	Not Listed
European/International Regulations	
European labeling in accordance with EC Directives	Not hazardous according to European directives
15.2 Chemical Safety Assessment	Not carried out

SECTION 16. Other information.

This information is true based on our present knowledge. However, EnviroLogix makes no representation of its accuracy or completeness. Persons receiving this information must exercise their independent judgment in determining the product's safety and suitability for its intended use. This document shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship

EHS Department
EnviroLogix Inc.

Codes:
H360 May damage fertility or the unborn child

SDS DB6 Dilution Buffer

Summary Guide for Approved Matrices									
Approved Matrix	**Add to Sample Extraction Vessel (in this order)**	**Then shake immediately**	**Clarify**	**Add Substrate Tablet to reaction tube**	**Add to the reaction tube and vortex**	**Add Reaction Tube to Incubator Set at 70 °C**	**Add DB6 and vortex**	**Rest at Room Temperature**	**Add Strip for**
! Freshly Ground Soft Red (MG1)	1. 20—-50 g sample 2. 5x volume of water* in mL per g of sample (ie. 20 g sample add 100 mL water) 3. Immediately shake vigorously to fully wet sample	30 seconds at highest speed (≥ 300 rpm) on shaker table or 30 seconds vigorously by hand	Filter (no longer than 2 minutes) or Centrifuge 30 sec. at 2000 x *g*	Add 1 Sprout Damage Substrate tablet to the reaction tube^	Add 300 µL of clarified extract and vortex at max speed for 15 seconds	Acclimate tube for 90 seconds	Immediately at 90 seconds: Add 300 µL of DB6 (lot specific) then Remove from incubator and vortex at max speed for 5 seconds	Rest reaction tube at room temperature for 30 seconds in a rack	3 min
! Freshly Ground Soft White Wheat (MG2)									
! Freshly Ground EU Soft Wheat (MG3)									
! Freshly Ground Brazil Wheat (MG4)									
! Freshly Ground Hard Red Wheat (MG5)									

Notes:

*Use deionized, distilled, or bottled water

^Use clean forceps, tweezers, or gloved hands

! Samples should be tested within 24 hours of being ground. When stored at room temperature, samples are stable for up to 24 hours after grinding.