




T-2test HPLC

Instruction Manual

VICAM®

A Waters Business

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1.1 INTENDED USER

T-2test™ HPLC method is a quantitative assay intended for use by trained customers in the food processing industry who need to test samples for the presence of T-2 toxin (T-2), with a method that is safe, simple, fast, and which works reproducibly and accurately.

1.2 PRINCIPLE

T-2 producing fungi commonly attack grains and can grow at temperatures from slightly above freezing to about 86°F (30°C). All domestic animals are susceptible to injury by dietary intake of T-2 in the range of a few ppm. In poultry, feed contaminated with 1.0 to 3.5 ppm of T-2 has produced lesions at the edges of the beaks, abnormal feathering in chicks, a drastic and sudden drop in egg production, eggs with thin shells, reduced weight gains, and mortality.

To measure T-2 levels, samples are prepared by mixing with an extraction solution, followed by blending and filtering. The extract is then applied to the T-2test column, which contains specific antibodies to T-2. At this stage, the T-2 binds to the antibody on the resin. The column is then washed to rid the resin of impurities from the extract. Then the T-2 is removed from the antibody by passing methanol through the column. The T-2 is derivatized and quantified by injection into an HPLC system.

1.3 APPLICABILITY

T-2test has been optimized for quantitative measurement of T-2 in wheat, corn and cereal grains (barley, oats, sorghum, rice).

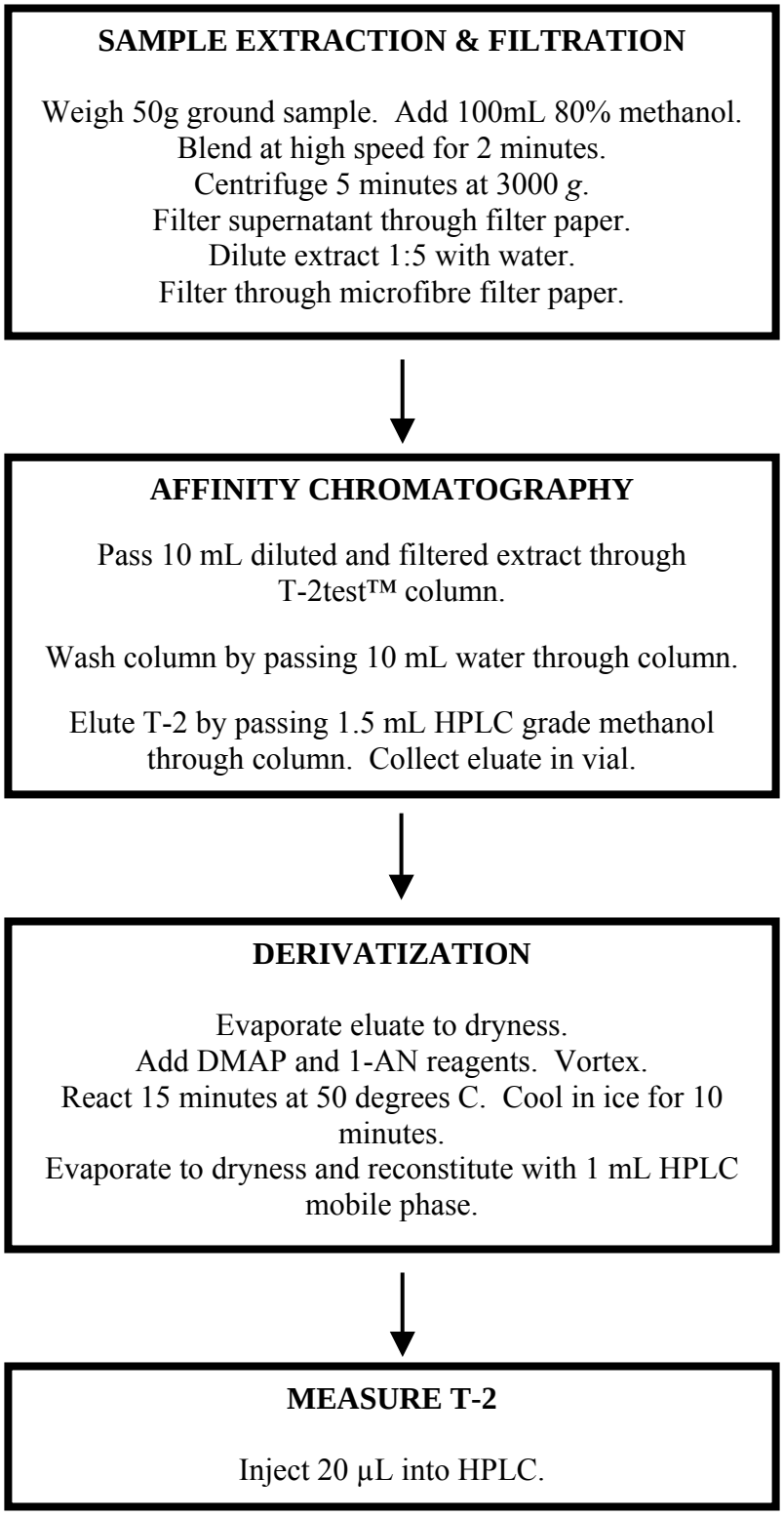
1.4 LIMITATIONS

This test has been designed for use with the procedure and reagents described on the following pages. Do not use materials beyond their expiration date. Deviation from these instructions may not yield optimum results.

1.5 SHELF LIFE AND STORAGE CONDITIONS

Store at room temperature (18-25 °C, 64-73 °F) until the expiration date printed on the label. Freezing may damage T-2test Resin.

1.6 T-2test™ CEREAL GRAINS PROCEDURE OVERVIEW



2.0 CLEANING EQUIPMENT

Wash blender jar, blender blade assembly, funnel and syringe barrel with soap and hot water. Rinse thoroughly with cold tap water and then dry completely.

3.1 MATERIALS REQUIRED
(These items will need to be reordered periodically)

Description	**Part #**
T-2test Columns (25)	G1028
4 mL silane treated amber vials (Supelco #27217)	
Methanol, HPLC Grade (4 x 4 L)	35016
4-dimethylaminopyridine (DMAP) 0.325 µg/µL in toluene (Sigma)	
1-anthroylnitrile (1-AN) 0.3 µg/µL in toluene *	
Acetonitrile, HPLC Grade	
Whatman #4 Filter Paper	
Whatman GF/A Filter Paper	
Distilled, reverse osmosis or deionized water	
Ice	

*1-anthroylnitrile (part number 017-12101**)** can be purchased from WAKO BioProducts at http://www.wako-chem.co.jp/english/.

Note: Be sure to use ultra-high purity toluene (i.e. for organic residue analysis).

3.2 EQUIPMENT REQUIRED

Description	**Part #**
Digital Scale with AC Adapter	20100
Commercial Blender with Stainless Steel Container	20200
Graduated Cylinder, 250 mL	20250
Graduated Cylinder, 50 mL	20050
Glass Blender Jar, 500 mL	20300
Wash Bottle, 500 mL (2)	20700
Disposable Plastic Beakers (25)	36010
Cuvette Rack	21010
Vortex Mixer	23040
Filter Funnels, 65 mm (4)	36020
Filter Funnel, 105 mm (4)	36022
Micro-pipettor, 1 mL adjustable	
Micro-pipettor, 50 µL	20604
Micro-pipette tips for 50 µL Micro-pipettor	20658
Micro-pipette tips for 1mL Adjustable Micro-pipettor	
Single or multiple position pump stand or vacuum manifold	21020
Centrifuge suitable for 3000g and 4 degrees C.	
Heating Block (50 degrees C.) and nitrogen tank	
Or speed-vac evaporator	
HPLC System (see method for details)	

4.0 T-2test™ HPLC PROCEDURE FOR CEREAL GRAINS (WHEAT, CORN, BARLEY, OATS, RICE AND SORGHUM)

1.0 HPLC Set up:

1.1 Column: Reverse phase, C18 (Waters Symmetry, 4.6 mm x 150 mm, 5μm column) preceded by a Rheodyne guard filter (0.5 μm).
1.2 Mobile phase: acetonitrile:water (80:20, v/v)
1.3 Flow rate: 1.0 mL/min
1.4 Injection volume: 20 μL
1.5 Fluorescence detector: Jasco FP-1520 fluorometric detector
1.6 Detection wavelength: Fluorescence: 381 nm excitation and 470 nm emission

2.0 Sample Extraction:

2.1 Weigh 50g ground sample into a blender jar.
2.2 Add 100 mL methanol:water (80:20, v/v).
2.3 Cover blender jar and blend at high speed for 2 minutes.
2.4 Centrifuge mixture for 5 minutes at 3000g* at 4°C.
2.5 Pour off supernatant and filter through Whatman #4 filter paper. Collect filtrate in a clean vessel.

3.0 Extract Dilution and Filtration:

3.1 Remove two end caps from T-2test affinity column.
3.2 Cut off tip of column top cap to use as a coupling. Attach column to outlet of 10 mL glass syringe barrel on pump stand.
3.3 Transfer 10 mL filtered extract into another clean vessel.
3.4 Dilute extract with 40 mL distilled, reverse osmosis, or deionized water. Mix well.
3.5 Filter dilute extract through microfibre filter (Whatman GF/A) into a clean vessel.
3.6 **Note**: For barley samples, an additional centrifugation (5 minutes, 3000g*) is necessary before filtration through the microfiber filter.

4.0 Column Chromatography:

4.1 Pass 10 mL diluted and filtered extract (equivalent to 1.0 g sample) completely through T-2test™ affinity column at a rate of about 1 drop/second until air comes through column.
4.2 Wash column by passing 10 mL of distilled, reverse osmosis, or deionized water through the column at a rate of 1-2 drops/second until air comes through the column.
4.3 Elute affinity column by passing 1.5 mL HPLC grade methanol through column at a rate of about 1 drop/second and collecting all of the sample eluate (1.5 mL) in a 4 mL silanized amber vial.
4.4 Evaporate to dryness under a nitrogen stream at approximately 50°C.

* The rpm value that corresponds to the specified g force will vary depending on the centrifuge rotor. For a JA18 rotor, 5250 rpm equals 3000g. Use a nomogram to identify the rpm corresponding to the specified g force for your centrifuge rotor. These are usually supplied with the rotor.

5.0 Derivatization Procedure

5.1 Add 50 µL of 4-dimethylaminopyridine (DMAP) solution into the vial followed by the addition of 50 µL of 1-anthroyl cyanide (1-AN) reagent. Mix by vortex for 1 minute.

5.2 Leave to react for 15 minutes at 50°C. Cool mixture in ice for approximately 10 minutes.

5.3 Evaporate to dryness the entire volume of the mixture under nitrogen stream at approximately 50°C and reconstitute with 1000 µL HPLC mobile phase.

6.0 HPLC Analysis

Inject 20 µL into the HPLC apparatus by full loop injection system.

7.0 Limit of Detection: 0.005 µg/g

8.0 Recovery: > 80% over the 0.05 - 1.5 µg/g range.

5.0 GENERAL PRECAUTIONS

Always use good, clean equipment and reagents (HPLC grade methanol for sample elution and distilled, reverse osmosis or deionized water for extraction and washing).

Maintain a steady flow rate of approximately 1-2 drops per second through the T-2test column during sample loading and elution.

Perform test from beginning to end without interruptions.

Silane treated vials, such as Supelco #27217 (4 mL silane treated amber glass vials), are recommended.

Be sure to use ultra-high purity toluene for preparing DMAP and 1-AN (i.e. for organic residue analysis). Interfering peaks may be observed with ACS toluene and toluene for HPLC.

6.0 DISPOSAL OF MATERIAL CONTAINING T-2

Transfer sample extract solutions and derivatization solutions into a liquid waste container for hazardous waste disposal.

7.0 TECHNICAL ASSISTANCE

If the desired results are not obtained, verify incubation times, temperatures and methods adhere to the procedure presented in this manual.

For assistance please contact your local distributor or VICAM Technical Services:

Phone:	800-338-4381	Canada, Mexico and the United States
	508-482-4935	all International and United States customers
Fax:	508-482-4972	
e-mail:	techservice@vicam.com	

8.0 LIABILITY

Only individuals with appropriate training should perform the test described in this instruction manual. Materials should be handled and disposed of properly.

The analytical methods described above have been developed by VICAM to be used exclusively with the reagents in this test. The user assumes all risk in using T-2test analytical procedures and products. VICAM makes no warranty of any kind, express or implied, other than that T-2test products conform to VICAM's printed specifications and quality control standards. VICAM will as its option repair or replace any product or part thereof which proves to be defective in workmanship or material. VICAM's undertaking to repair or replace such products is exclusive and is in lieu of all warranties whether written, oral expressed, or implied, including any implied warranty of merchantability or fitness for a particular purpose. VICAM shall have no liability for anticipated or lost profits or any loss, inconvenience or damage whether direct, incidental, consequential or otherwise, to person or property, or for strict liability or negligence arising from or in connection with the use of these assay procedure of T-2test product.

Worldwide patents and trademarks protect all VICAM products.

9.0 REFERENCES

Pascale, M., Haidukowski, M., Visconti, A., Journal of Chromatography A, "Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile", **989** (2003) 257-264.

10.0 ORDERING INFORMATION

To place an order contact your local VICAM distributor or VICAM at:

Phone:	877-228-4244	United States and Canada
	800-338-4381	Mexico
	417-725-6588	all International and United States customers
Fax:	417-725-6102	
e-mail:	vicam@vicam.com	