

CHARM SCIENCES, INC.
ROSA WETS5 AFLATOXIN QUANTITATIVE TEST

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GENERAL INFORMATION

ROSA WET-S5 Aflatoxin Quantitative Test is an immunoreceptor assay utilizing ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET) that eliminates the use of organic solvents (methanol, ethanol, etc.). WET uses a non-hazardous extraction powder added to the sample followed by water to extract aflatoxin into the aqueous solvent. Aflatoxin interacts with colored beads in the lateral flow test strip and the color intensity in the test and control zones is measured by the ROSA-M Reader or Charm EZ-M reader and displayed as parts per billion (ppb) aflatoxin.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	<i>Charm Sciences, Inc. 978-687-9200</i>
Test Kit Name:	ROSA WET-S5 Aflatoxin Quantitative Test
Product Number:	LF-AFQ-WET-S5
Effective Date of Instructions:	9/11/2015
Instructions Revision Number	1
Conformance Range:	5 – 100 ppb
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	Yes
Approved Commodities:	Corn, barley, corn flour, corn grits, corn meal, corn screenings, corn/soy blend, distillers dried grain with solubles (DDGS), hominy, oats, rough rice, sorghum, soybeans, and wheat.
Extraction method:	Shake vigorously 50 gram sample with one (1) packet of WET-S Extraction Powder and 150 milliliters (mL) of deionized or distilled water for 1.5 minutes. For barley, corn/soy blend, DDGS, and soybeans shake vigorously 50 gram sample with two (2) packets of WET-S Extraction Powder and 250 mL deionized or distilled water for 1.5 minutes.
Test Format:	Lateral flow strip
Detection Method:	ROSA-M Reader, Model # LF-ROSAREADER-M-NB and Charm EZ-M reader, Model # LF-ROSA-EZ-M

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

a. Test Strips:

Remove from the container only the number of test strips to be used in one day, document time of removal. Keep these test strips at room temperature during daily use for up to 12 hours and unused test strips should be discarded.

b. AFQ Dilution Buffer:

- (1) Dispense buffer into a clean micro-centrifuge tube and label for each sample to be tested.
- (2) Use pre-dispensed buffer tubes and buffer solution at room temperature (18 °C to 30 °C).

c. EP Control:

Reconstitute one packet WET-S Extraction Powder in 150 mL deionized or distilled water by gently swirling until extraction powder is dissolved.

d. Negative Control

Prepare negative control by adding 300 microliters (µL) EP Control to 600 µL AFQ Dilution Buffer in a clean micro-centrifuge tube, cap, mix, and label.

e. Positive Control

Reconstitute the dry positive control by adding 1.0 mL EP Control followed by 2.0 mL AFQ Dilution Buffer. Shake well and allow to stand at room temperature for 10 minutes. Mix again before use.

f. Reader and Test Strip Performance Testing:

- (1) Equipment Setup
 - (a) **ROSA-M Reader:** Enter performance mode in ROSA-M Reader by selecting MYCO2 channel in 3-line mode (MYCO2 flashing) and sequentially pressing ESC, 5, ENTER. Follow ROSA-M Reader prompts to test calibration strips (LOWCAL and HIGHCAL) and controls (NEGCONTROL and POSCONTROL).
 - (b) **Charm EZ-M reader:** Enter performance mode in Charm EZ-M reader by selecting Perf. Mon. from the Main Menu, followed by Perf. Test. Follow the reader prompts to test calibration strips (LO CAL and HI CAL) and controls (NEG CTRL and POS CTRL). Select AFQ-WETS5 from the TESTS list if prompted.
- (2) Test calibration strips daily to verify performance of ROSA-M Reader or Charm EZ-M reader. Calibration strips must test/perform in the specified ranges.
- (3) Test negative control and positive control weekly to verify test strip performance. Valid control ranges are:
 - (a) Negative Control: less than 3 ppb
 - (b) Positive Control: 12 to 28 ppb

If calibration strips or controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance. Notify your monitoring field office or TSD with any documented information for quality control purposes.

g. ROSA Incubator:

ROSA Incubator must be clean and level. The ROSA Incubator temperature must be at 45 ± 1 °C (the temperature indicator should match the incubator temperature).

SAMPLE PREPARATION AND EXTRACTION PROCEDURES

a. 3:1 Extraction Procedure for corn, corn flour, corn grits, corn meal, corn screenings, hominy, oats, rough rice, sorghum, and wheat:

- (1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
- (2) Add contents of one (1) packet of WET-S Extraction Powder.
- (3) Add 150 mL deionized or distilled water.
- (4) Shake vigorously for 1.5 minutes by hand.
- (5) Allow sample to settle for 1 minute to obtain settled extract (can be used only for next 30 minutes).
- (6) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).
- (7) Pipet 600 μ L AFQ Dilution Buffer into a clean micro-centrifuge tube.
- (8) Pipet 300 μ L centrifuged sample extract to micro-centrifuge tube containing 600 μ L AFQ Dilution Buffer, cap, mix, and label. This sample is the Diluted Extract.
- (9) Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator).

b. 5:1 Extraction Procedure for barley, corn/soy blend, DDGS, and soybeans:

- (1) Weigh 50.0 ± 0.2 grams ground samples into a clean extraction container.
- (2) Add contents of two (2) packets of WET-S Extraction Powder.
- (3) Add 250 mL deionized or distilled water.
- (4) Shake vigorously for 1.5 minutes.
- (5) Allow sample to settle for 1 minute to obtain settled extract (can be used only for next 30 minutes).
- (6) Transfer 1 to 1.5 mL settled extract into a clean micro-centrifuge tube, label, and centrifuge for 10 seconds (can be used only for next 2 hours).
- (7) Pipet 400 μ L AFQ Dilution Buffer into a clean micro-centrifuge tube.
- (8) Pipet 500 μ L centrifuged sample extract to micro-centrifuge tube containing 400 μ L AFQ Dilution Buffer, cap, mix, and label. This sample is the Diluted Extract.
- (9) Repeat for additional samples (up to four (4) samples for each quad ROSA Incubator).

TEST PROCEDURES

a. Sample Analysis:

- (1) Check that the ROSA Incubator temperature is 45 ± 1 °C.
- (2) Label test strip(s) to identify sample.
- (3) Place test strip in the ROSA Incubator with the flat side facing upward.
- (4) Hold the test strip flat in the ROSA Incubator and use tab to expose sample compartment by peeling tape back to “Peel to Here” line.

Avoid lifting the test strip and sponge under tape and bending back the white wick and sponge under the tape.

- (5) Hold the pipet vertically and slowly pipet 300 µL test sample (diluted extract or control) into the sample compartment at the ROSA Incubator line.
- (6) Reseal the tape over the sample pad compartment.

NOTE: When performing multiple tests using a ROSA Incubator:

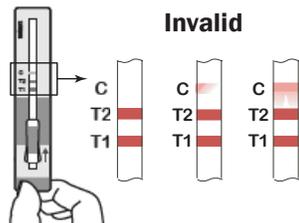
- (a) Peel, pipet, and reseal before starting next strip.
- (b) Complete all test strips (up to four (4) with quad incubator) within 1 minute.
- (7) Close lid on the ROSA Incubator.
- (8) Incubate for 5 minutes.
- (9) Remove strip from the ROSA Incubator.

Do not squeeze sample compartment. Hold test strip vertically with sample compartment in the down position until interpreted.

- (a) Wipe foreign matter (dust, etc.) from the test strip(s).
- (b) Inspect and read test strip(s) within 60 seconds of incubation completion. When running multiple test strips in the ROSA Incubator, remove one strip for visual inspection and interpretation at a time.
- (c) Lower ROSA Incubator lid; do not re-latch.

b. Visual Inspection:

- (1) The test strip is **INVALID** if any of the following are observed:
 - (a) C (Control) line is missing.
 - (b) T1, T2 (Test) or C line is smeared or uneven.
 - (c) T1, T2, or C line is obscured by diluted extract or control.
 - (d) Beads do not flow past T1, T2 or C lines.



- (2) Do not put INVALID test strips in the ROSA-M Reader or Charm EZ-M reader.
- (3) If test strip is INVALID, re-test the diluted extract or control.

c. Interpretation:

(1) ROSA-M Reader

- (a) Insert a clean and valid test strip into the ROSA-M Reader. Slide the strip into the slot with the sample compartment in the up position until it stops.
- (b) Read results on MYCO2 channel in 3-line mode (MYCO2 flashing) using the appropriate MATRIX. If desired, enter Sample and/or Operator. Press ENTER to read.

- **MATRIX 00:** Diluted Extract for 5 to 100 ppb quantitation for barley, corn, corn grits, corn meal, hominy, and soybeans.
- **MATRIX 01:** Diluted Extract for 5 to 100 ppb quantitation for corn flour, corn screenings, corn/soy blend, DDGS, oats, rough rice, sorghum, and wheat.
- **MATRIX 02:** Supplemental Diluted Extract for 100 to 1000 ppb quantitation (corn only).

- (c) **READING:** The number displayed is the concentration of aflatoxin (ppb) in the sample.

A Diluted Extract READING greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution.

An applicant can request a supplemental analysis (corn only) option to report test results above the Diluted Extract sensitivity range of 100 ppb. See Supplement Analysis procedures for more information.

(2) Charm EZ-M reader (Read only mode)

- (a) Insert a clean and valid test strip into the Charm EZ-M reader. Slide the strip into the slot with the sample compartment in the down position until it stops.
- (b) Read results on AFQ-WETS5 from the TESTS list with COMMODITY and DILUTION selected for sample. If desired, enter OPERATOR ID, SAMPLE ID, and/or LOT NUMBER. Close door to read.

- **DE:** Diluted Extract for 5 to 100 ppb quantitation (all commodities).
- **SUPP DE:** Supplemental Diluted Extract for 100 to 1000 ppb quantitation (corn only).

- (c) **READING:** The number displayed is the concentration of aflatoxin (ppb) in the sample.

A Diluted Extract **READING** greater than 100 ppb indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution.

An applicant can request a supplemental analysis (corn only) option to report test results above the Diluted Extract sensitivity range of 100 ppb. See Supplement Analysis procedures for more information.

SUPPLEMENTAL ANALYSIS

Supplemental analysis (corn only) is a procedure followed when a result is observed above the upper limit of the concentration range used in GIPSA's test kit performance evaluation.

The range for performance evaluation of quantitative aflatoxin test kits is 5 – 100 ppb. Therefore, supplemental analysis would be performed for a result above 100 ppb. In supplemental analysis, the extract is diluted so the resulting concentration is between the lower and upper limits of the test kit evaluation range (i.e., 5 – 100 ppb for aflatoxins), and a correction for dilution is applied to derive at the final result. For this test kit, the appropriate calibration setting is selected for automatic correction for the supplemental dilution performed.

Supplemental analysis is performed only at the request of the applicant.

Preparation and Assay of Supplemental Diluted Extract for 100 to 1000 ppb aflatoxin.

- (1) Prepare Diluted Extract according to Sample Preparation and Extraction Procedures.
- (2) Prepare Supplemental Diluted Extract from the Diluted Extract.
 - (a) Pipet 1.0 mL AFQ Dilution Buffer into a clean micro-centrifuge tube.
 - (b) Pipet 100 μ L Diluted Extract to micro-centrifuge tube containing 1.0 mL AFQ Dilution Buffer, cap, mix, and label. This sample is the Supplemental Diluted Extract.
- (3) Repeat for additional samples.
- (4) Use Supplemental Diluted Extract as test sample in Sample Analysis found in Test Procedures section.
- (5) Inspect and interpret the test strip as directed in Test Procedures section.

Valid Supplemental Diluted Extract final result must be within 53 to 1000 ppb detection range of the sample dilution.

A final result less than 53 ppb is indicative of a problem, and troubleshooting is needed. Verify the procedure is being followed properly. Perform the procedure for the Diluted Extract (non-supplemental analysis) and only perform the supplemental analysis again if the value is greater than 100 ppb.

A Supplemental Diluted Extract **READING** of “+1000 ppb” indicates that the concentration of the sample is greater than the sensitivity range of the sample dilution. Report test results as greater than 1000 ppb on the work record and certify “Aflatoxin exceeds 1000 ppb”.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@usda.gov).

STORAGE CONDITIONS AND PRECAUTIONS

a. Storage Conditions:

- (1) Store test strips refrigerated in tightly closed supplied container.
- (2) Store dilution buffer bottle and pre-dispensed micro-centrifuge tubes refrigerated.
- (3) Store WET-S Extraction Powder at room temperature in supplied packet.
- (4) Store reconstituted EP Control refrigerated (0 °C to 7 °C) for up to one week or aliquot (at least 1.5 ml) to clean micro-centrifuge tubes, label, and freeze within six hours of reconstitution (-15 °C or below) for up to two months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed EP Control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.
- (5) Store reconstituted positive control refrigerated (0 °C to 7 °C) for up to one week or aliquot (at least 1.5 ml) to clean micro-centrifuge tubes, label, and freeze within six hours of reconstitution (-15 °C or below) for up to two months. Thaw slowly (overnight in refrigerator or with cool water) and shake well before use. Store thawed positive control refrigerated and use within 24 hours of thawing; DO NOT REFREEZE.

b. Precautions:

- (1) Test Strips
 - (a) To open test strip canister, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.
 - (b) In high humidity, limit condensation by opening container after it has warmed to room temperature, estimated between 20 to 30 minutes from the time the container was removed from the refrigerator.
 - (c) Inspect/verify desiccant indicator. Beads inside desiccant packets should be blue. Do not use test strips if the blue beads have turned purple or pink.
- (2) Use AFQ Dilution Buffer supplied with each test kit only.
- (3) WET-S Extraction Powder
 - (a) WET-S Extraction Powder is non-hazardous and may be disposed as normal waste.
 - (b) Do not open WET-S Extraction Powder until ready to use.
- (4) Do not use the test kits beyond the noted expiration date.
- (5) Debris on test strips may alter the reader optics. Keep equipment clean. Wipe dust and liquid off test strips before inserting into reader.
- (6) ROSA Incubator must be clean and level. ROSA Incubator temperature must be 45 ± 1 °C. The temperature indicator should match the ROSA Incubator temperature. A daily

thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched unless performing test procedure. ROSA Incubator may take 10 minutes to reach proper temperature depending on ambient temperature.

EQUIPMENT AND SUPPLIES

a. Test Strips

- (1) LF-AFQ-WETS5-20K
 - (a) 1 container of 20 AFQ-WETS5 test strips
 - (b) 1 Aflatoxin B1 Positive Control
 - (c) 1 AFQ Dilution Buffer
- (2) LF-AFQ-WETS5-100K
 - (a) 1 container of 100 AFQ-WETS5 test strips
 - (b) 1 Aflatoxin B1 Positive Control
 - (c) 1 AFQ Dilution Buffer
- (3) LF-AFQ-WETS5-500K
 - (a) 5 containers of 100 AFQ-WETS5 test strips
 - (b) 5 Aflatoxin B1 Positive Controls
 - (c) 5 AFQ Dilution Buffers

b. WET-S Extraction Powder

- (1) LF-WET-EXTS-50G-20: WET-S Extraction Powder for 50 gram sample (20/pack)
- (2) LF-WET-EXTS-50G-100: WET-S Extraction Powder for 50 gram sample (100/pack)

c. Materials required but not provided

- (1) 300 μ L pipet and pipet tips
- (2) 100 to 1000 μ L variable volume pipet and pipet tips
- (3) 250 mL graduated cylinder
- (4) Balance
- (5) Deionized or distilled water
- (6) Micro-centrifuge tubes
- (7) Mini-centrifuge
- (8) ROSA Incubator
- (9) ROSA-M Reader or Charm EZ-M reader
- (10) Printer for ROSA-M Reader or Charm EZ-M reader (optional)
- (11) Sample grinder
- (12) Sample extraction containers

- (13) Storage bottle
- (14) Transfer pipets (optional)

REVISION HISTORY

Revision 1 (9/11/2015)

- Flow charts have been incorporated into the instruction.

Revision 0 (2/6/2015)

FLOW CHARTS

a. Flow chart for 3:1 Extraction Procedure and Assay for corn, corn flour, corn grits, corn meal, corn screenings, hominy, oats, rough rice, sorghum, and wheat:

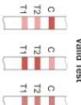
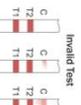
Refer to GIPSA Test Kit Instructions for Complete Test Procedure

ROSA® WET®-S5 Aflatoxin Quantitative Test Flow Chart - 3:1 Extraction

Approved Commodity:
 Corn, Corn Flour, Corn Grits, Corn Meal, Corn Screenings, Hominy, Oats, Rough Rice, Sorghum, Wheat

See Approved Commodity Below

Quantitation Ranges: 5 to 100 ppb to 100 to 1000 ppb

Sample Preparation	Test Procedure	Read Result																				
<p>(1) Weight Ground sample 50.0 ± 0.2 g</p> <p>(2) Add WET-S Extraction Powder 1 Packet for 50 g sample</p> <p>(3) Add Solvent Deionized or Distilled Water 150 mL</p> <p>(4) Extract Shake vigorously for 1.5 minutes</p> <p>(5) Clarify Allow extract to settle for 1 minute Centrifuge extract for 10 seconds</p> <p>(6) Dilute Prepare Diluted Extract.</p> <div style="text-align: center;"> <p>300 µL Centrifuged Extract + 600 µL AFQ Dilution Buffer</p> <p>Mix</p> <p>Diluted Extract</p> </div>	<p>(1)  Place test strip in ROSA-M Incubator.</p> <p>(2)  Peel tape. Pipet 300 µL Diluted Extract into sample compartment. Reseal tape.</p> <p>(3)  Close lid. Incubate for 5 minutes.</p>	<p>(1) Inspect test strip</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>Valid Test</p>  </div> <div style="text-align: center;"> <p>Invalid Test</p>  </div> </div> <p>(2) Read result with ROSA-M Reader or Charm EZ-M reader ROSA-M Reader: Select MYCO2 channel in 3-line mode (blinking) and appropriate MATRIX. Charm EZ-M reader: Select appropriate test (AFQ-WETS5), commodity and dilution if prompted.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Commodity</th> <th>Sample</th> <th>Charm EZ-M Reader</th> <th>ROSA-M Reader</th> <th>Quantitation Range</th> </tr> </thead> <tbody> <tr> <td>Corn, Corn Grits, Corn Meal, Hominy</td> <td>Diluted Extract</td> <td>DILUTION DE</td> <td>MATRIX 00</td> <td>5 to 100 ppb</td> </tr> <tr> <td>Corn Flour, Corn Screenings, Oats, Rough Rice, Sorghum, Wheat</td> <td>Diluted Extract</td> <td>DILUTION DE</td> <td>MATRIX 01</td> <td>5 to 100 ppb</td> </tr> <tr> <td>Corn</td> <td>Supplemental Diluted Extract</td> <td>DILUTION SUPP DE</td> <td>MATRIX 02</td> <td>100 to 1000 ppb</td> </tr> </tbody> </table>	Commodity	Sample	Charm EZ-M Reader	ROSA-M Reader	Quantitation Range	Corn, Corn Grits, Corn Meal, Hominy	Diluted Extract	DILUTION DE	MATRIX 00	5 to 100 ppb	Corn Flour, Corn Screenings, Oats, Rough Rice, Sorghum, Wheat	Diluted Extract	DILUTION DE	MATRIX 01	5 to 100 ppb	Corn	Supplemental Diluted Extract	DILUTION SUPP DE	MATRIX 02	100 to 1000 ppb
Commodity	Sample	Charm EZ-M Reader	ROSA-M Reader	Quantitation Range																		
Corn, Corn Grits, Corn Meal, Hominy	Diluted Extract	DILUTION DE	MATRIX 00	5 to 100 ppb																		
Corn Flour, Corn Screenings, Oats, Rough Rice, Sorghum, Wheat	Diluted Extract	DILUTION DE	MATRIX 01	5 to 100 ppb																		
Corn	Supplemental Diluted Extract	DILUTION SUPP DE	MATRIX 02	100 to 1000 ppb																		
For quantitation of 100 to 1000 ppb: For Corn Only		<p>(1) Prepare Supplemental Diluted Extract</p> <div style="text-align: center;"> <p>100 µL Diluted Extract + 1000 µL AFQ Dilution Buffer</p> <p>Mix</p> <p>Supplemental Diluted Extract</p> </div> <p>(2) Repeat Test Procedure (steps 1, 2, 3) with Supplemental Diluted Extract</p> <p>(3) Read Results (valid range is 53 to 1000 ppb)</p>																				



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b. Flow chart for 5:1 Extraction Procedure and Assay for barley, corn/soy blend, DDGS, and soybeans:

Refer to GIPSA Test Kit Instructions for Complete Test Procedure

ROSA® WET®-SS Aflatoxin Quantitative Test Flow Chart - 5:1 Extraction

Approved Commodity:
Barley, Corn/Soy Blend, DDGS, Soybeans

See Approved Commodity Below

Quantitation Range: 5 to 100 ppb

Sample Preparation

Test Procedure

Read Result

(1) Weigh
 50.0 ± 0.2 g
 Ground sample

(2) Add WET-S Extraction Powder
 2 Packets for 50 g sample

(3) Add Solvent
 Deionized or Distilled Water
 250 mL

(4) Extract
 Shake vigorously for
 1.5 minutes

(5) Clarify
 Allow extract to settle
 for 1 minute
 Centrifuge extract for
 10 seconds

(6) Dilute
 Prepare Diluted Extract.

(1)
 Place test strip in ROSA Incubator.

(2)
 Peel tape.
 Pipet 300 µL Diluted Extract
 into sample compartment.
 Reseal tape.

(3)
 Close lid.
 Incubate for 5 minutes.

(1) Inspect test strip

Valid Test

Invalid Test

(2) Read result with ROSA-M Reader or Charm EZ-M reader
 ROSA-M Reader: Select MYCO2 channel in 3-line mode (blinking) and appropriate MATRIX.
 Charm EZ-M reader: Select appropriate test (AFQ-WETS5), commodity and dilution if prompted.

Commodity	Sample	Charm EZ-M Reader DILUTION DE	ROSA-M Reader MATRIX 00	Quantitation Range
Barley, Soybeans	Diluted Extract	DILUTION DE	MATRIX 00	5 to 100 ppb
Corn/Soy Blend, DDGS	Diluted Extract	DILUTION DE	MATRIX 01	5 to 100 ppb

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