

### ACETIC ACID

**New automation option:**  
ChemWell *Auto-Prep* tests (refer to **NOTES**)

**Product #:** **AA-F60 (30 Tests)**  
**AA-F150 (75 Tests)**  
**AA-F500 (250 Tests)**

**Enzymatic, UV-Method**

#### INTENDED USE

**Acetic Acid FLEX-REAGENT™** is intended for measuring acetic acid (acetate) concentrations in wine.

#### KIT CONTENTS

	<b>30T</b>	<b>75T</b>	<b>250T</b>
Opti-Buffer Solution #1	20 mL	50 mL	170 mL
Co-Enzyme Powder #2	4 mL	10 mL	2x17 mL
MDH/CS Solution #3	1.3 mL	3.3 mL	2x5.5 mL
ACS Enzyme Powder #4a	1.3 mL	3.3 mL	2x5.5 mL
ACS Diluent #4b	1.4 mL	3.4 mL	12 mL
Acetic Acid 0.4G/L Std*	1 mL	1 mL	5 mL

\* The **0.4G/L level standard** is now supplied to improve calibration and monitoring at levels critical for winemaking. Previous kits contained 0.15G/L Standard; alternate standards and a 5-Level Set are available from Unitech Scientific. No change is required to Manual Calculations (extinction coefficient method) below.

#### SYSTEM REQUIREMENT

Spectrophotometer should be capable of reading 340 nm absorbance over a 0-2 A range with a 1 cm light path.

#### SAMPLES

If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples.

#### REAGENTS AND STORAGE

Kit contents are stable through the labeled expiration date when stored at 2-8 °C. The following are **ready to use**: Opti-Buffer, MDH/CS Solution, and Standard.

#### ASSAY PREPARATION

##### Dissolve Reagent Powders

- Dissolve a bottle of **Co-Enzyme Powder (#2)** with deionized water per label. Stable **3 months** at 2-8 °C. Note expiry date on label.
- Dissolve a bottle of **ACS Enzyme Powder (#4a)** with **ACS Diluent (#4b)** per label. Stable **3 months** at 2-8 °C. Note expiry date on label.

**Prepare Working Reagent**, (Traditional) manual procedure<sup>1</sup> as below, for the number of cuvettes (blank, standard and wine samples) in your assay:

	<b>Volumes Required</b>	
	<b>Each test</b>	<b>3 Cuvettes</b>
Opti-Buffer Solution #1	0.67mL	2.0mL
Co-Enzyme Solution #2	0.133mL	0.4mL
Deionized Water	1.33mL	4.0 mL
<b>Working Rgt (Approx.)</b>	<b>2mL</b>	<b>6mL</b>

Working Rgt is stable for 8-hours refrigerated, prepare just prior to testing. Allow reagent to reach room temperature before use.

**Note 1.** Refer to Appendix for alternative W-Rgt Prep and Test Procedures:

- Extended Range** Working Reagent & Manual procedure
- ChemWell-for-Wine™** Working Reagent

#### TESTING PROCEDURE

Pipet each solution into cuvettes, as shown:

	<b>Blank</b>	<b>Sample(s)</b>
<b>1. D.I. Water</b>	<b>20µL</b>	
<b>2. Sample</b>		<b>20µL</b>
<b>3. Working Rgt</b>	<b>2.0mL<sup>2</sup></b>	<b>2.0mL</b>
Mix cuvettes, incubate 3 minutes. Zero spectrophotometer (340 nm) with Reagent Blank Read A <sub>0</sub> (Initial ABS)		
<b>4. Solution #3</b>	<b>40µL<sup>3</sup></b>	<b>40µL</b>
Mix cuvettes, incubate 3 minutes, Read A <sub>1</sub>		
<b>5. Solution #4</b>	<b>40µL<sup>3</sup></b>	<b>40µL</b>
Mix cuvettes, incubate 20-30 minutes, Read A <sub>2</sub> <b>Note 2.</b> 2.0mL = 2000µL <b>Note 3.</b> 40µL (or 1 drop)		

The testing range is up to 0.75 G/L. If a calculated result is over-range, dilute sample and retest, multiply this test result by the dilution factor. (Inquire for Extended Range procedure & calculations for testing without dilution to 1.5 G/L.)

**CALCULATIONS:** The G/L result is calculated using a linearizing formula.

##### 1. Flex Calculator™ Method:

Our online "Flex Calculator™ Acetic Acid" spreadsheet at <http://unitechscientific.com/calculators.htm> is available for download. ΔA<sub>AcAc</sub> and G/L values will be calculated automatically.

##### 2. Manual Calculation Method - Calculate ΔA<sub>AcAc</sub> and G/L:

$$a) \Delta A_{AcAc} = \left[ \frac{(A_2 - A_0)_{sample} - (A_1 - A_0)_{sample}^2}{(A_2 - A_0)_{sample}} \right] - \left[ \frac{(A_2 - A_0)_{blank} - (A_1 - A_0)_{blank}^2}{(A_2 - A_0)_{blank}} \right]$$

$$b) \text{Acetic Acid G/L} = 1.001 \Delta A_{AcAc} \quad \text{For details, see APPENDIX.}$$

#### QUALITY CONTROL

Assay the acetic acid standard provided to monitor reaction completion and assay performance. In a valid assay, recovered value is +/- 15% of the labeled value. Factors that may affect performance include instrument function, temperature, glassware cleanliness, and pipetting accuracy (use calibrated micro-pipet). A 5-Level AA Standards Kit is available from Unitech Scientific.

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## APPENDIX

### NOTES FOR MANUAL CALCULATIONS Extinction Coefficient:

(G/L results are based on factor and the  $\Delta A_{AcAc}$  values above.  
Compare standard result with known value to verify recovery.)  
Factor is derived as follows:

$$G/L = \frac{MW \times T.V. \times d.f. \times \Delta A_{Acetic\ Acid}}{(\epsilon)(P)(1000mg/g)(SV)} = 1.001 \Delta A_{AcAc}$$

MW = 60.05 G/mole

TV = total reaction vol. (2 + 0.02 + 0.04 + 0.04) = 2.10 mL

SV = sample volume (0.020 mL)

$\epsilon$  (absorptivity of NADP) = 6.3 @ 334-340nm [or 3.4 @ 365nm]

P = 1 cm light path

d.f. = dilution factor

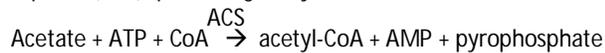
Adjust calculations if alternate Working Reagent & Sample Volumes are used.

### SPECIFICITY

This method is specific for acetate in wine samples; ethyl acetate is largely undetected by enzymatic analysis. For samples with unusually high ethyl acetate content, a correction method is described in our "Flex Calculator™ Acetic Acid" spreadsheet.

### METHODOLOGY & CHEMICAL PRINCIPLES

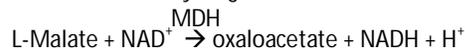
The enzyme acetyl-CoA synthetase (ACS) catalyzes the reaction of AA (acetate) with coenzyme A (CoA) in the presence of adenosine-5'-triphosphate (ATP), producing acetyl-CoA.<sup>1</sup>



Citrate synthase (CS) catalyzes the reaction of acetyl-CoA and oxaloacetate to form citrate.



Oxaloacetate, consumed in this reaction, is formed from L-Malic acid in the presence of malate dehydrogenase as follows:



The increase in NADH concentration is measured at 340nm and is the basis for calculation of AA concentration in the sample.

### SIGNIFICANCE OF MEASUREMENTS

Acetic acid is the primary acid formed during wine spoilage; legal limits vary by wine region. Volatile acidity determined by distillation consists primarily of AA, propionic and lactic acids; specific (enzymatic) acetic acid values are 90-98% of volatile acidity values in modern winemaking practice.

Ethyl acetate is typically determined using GC-FID, and is largely undetected by enzymatic and distillation analysis.

### EXTENDED RANGE, MANUAL - Working Reagent

Prepare working reagent just prior to testing (stable 2-hr. at 5°C):

	<b>3T</b>
Opti-Buffer Solution #1	2.0 mL
MDH/CS Solution #3	0.15 mL
Co Enzyme Solution #2	0.40 mL
Deionized Water	<u>4.0 mL</u>
WRgt (Approx. Total)	6.5 mL

### EXTENDED RANGE, MANUAL - Testing Procedure

This procedure requires a 0.9 G/L Acetic Acid Standard (available from Unitech Scientific) – in addition to the 0.4 G/L Standard provided. The extended testing range is 0 to 1.5 G/L. If a calculated result is over-range, dilute sample, retest, and multiply result by this factor.

### TESTING PROCEDURE Extended Range Working Reagent

	<b>Std. #1</b>	<b>Std. #2</b>	<b>Samples</b>
<b>1. 0.4 G/L Standard</b>	20µL		
<b>0.9 G/L Standard</b>		20µL	
<b>Samples</b>			20µL
<b>2. Working Reagent</b>	2.0 mL (= 2000µL)		
	Mix cuvettes, wait 3 min.		
	Zero spec. (340 nm) w/ DI Water		
	<b>Read A<sub>0</sub></b> (Initial ABS)		
<b>3. ACS Soln. #4</b>	40µL (= 1 drop)		
	Mix cuvettes, incubate 20-min / 37C		
	<b>Read A<sub>1</sub></b> (Final ABS)		

CALCULATE Acetic G/L Results. Save online '2-Std Calculator' (<http://www.unitechscientific.com/calculators.htm>) and enter **A<sub>0</sub>** & **A<sub>1</sub>** ABS values for Standards & Samples.

CHEMWELL for WINE™ - Working Reagent (Stable 5-days at 5°C)  
Automate Working Reagent preparation: \* Use **AP [=Auto-Prep]** protocols; This option is detailed below.

1. Prepare working reagent:

	<b>20T</b>	<b>55T</b>
Opti-Buffer Solution #1	2.0 mL	4.0 mL
MDH/CS Solution #3	0.13 mL	0.26 mL
Deionized Water	<u>4.0 mL</u>	<u>8.0 mL</u>
WRgt (Approx. Total)	6.1 mL	12.2 mL

(# of Tests accounts for Reagent Bottle dead volume)

2. Dissolve Co-Enzyme and ACS powder components as described in ASSAY PREPARATION section, Page 1. Note: these instructions assume you are using that the **latest CW software & Acetic protocol - no dilution** of ACS Enzyme needed. (If your lab is still using **diluted ACS** [stable 1 day at 5°C] see Note \*\*.)

**ChemWell-T for Wine (no ACS dilution required)** Place (undiluted) ACS enzyme directly in Reagent Rack.

NOTES: \* **AP [=Auto-Prep]** protocols for ChemWell & CW-T for Wine. Simply place each kit component directly in Reagent Rack. **With AP protocols, your CW will:**

- ✓ Automatically prepare Working Reagent in each cuvette
- ✓ Optimize your reagent usage & improve lab work flow.

With Reagent Cooling and **AP protocols**, UniFLEX reagents are stable in your ChemWell reagent rack for months.

Contact Unitech Scientific Tech Service for your new **AP protocol**

\*\* **The latest** (build 883 or higher) ChemWell for Wine **software** uses **undiluted ACS Enzyme** Solution, which is Stable 3-Mo 5C.

Place ACS enzyme directly in Reagent Rack.

Contact Unitech Scientific for **latest software & Acetic protocol**.

**The latest ChemWell software features:**

- ✓ (undiluted) ACS Enzyme Solution is loaded in Reagent Rack - get 3 months ACS Enzyme stability On Board your CW [Reagent Cooling required].
- ✓ Faster set-up, easier

### TRADEMARKS:

"ChemWell for Wine", "Flex Calculator", "FLEX Reagent" are Trademarks of Unitech Scientific

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