UNITECH SCIENTIFIC CALIFORNIA, U.S.A. FLEX-REAGENTTM

ACETALDEHYDE Procedure Change – Start Enzyme Method

Product #: ACE-F40 (20 Tests)

Enzymatic, UV- Method

INTENDED USE

This reagent is intended for measurement of Acetaldehyde concentrations in wine.

A OF F 40

KIT CONTENTS

	ACE F40
Buffer	40mL
NAD	2 vials
Aldehyde Dehydrogenase	2 vials
Enzyme Diluent	2x11 mL
ACE 0.1 G/L	2 mL
	Buffer NAD Aldehyde Dehydrogenase Enzyme Diluent ACE 0.1 G/L

SYSTEM REQUIREMENT

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

SAMPLES

Dilution: The measuring range, up to 0.12 G/L, is appropriate for acetaldehyde concentrations in table wine (red 0.030 G/L, white 0.080 G/L). If the Initial Absorbance (AINITIAL) exceeds 1.2 A, or if the calculated result is exceeds 0.12 G/L, dilute the sample & multiply result by dilution factor.

Clarification: If wine samples are visually clear, no sample pretreatment is needed. Filter or centrifuge turbid samples, e.g. juice, must or fermentation samples. Note that each lab should verify the need to decolorize red wine (e.g. with PVP at 1%).

REAGENTS AND STORAGE

Kit contents are stable through the labeled expiration date. Opened reagents are stable after opening if the bottles are protected from direct light and tightly closed. Store refrigerated at +2-8C.

REAGENT PREPARATION - Manual

Prepare Reagents as follows, then mix gently until completely dissolved; let sit 5 minutes at +15-25C before use .:

Reagent Solution #2: Reconstitute one vial of Reagent 2 with 20 mL of Reagent 1 (buffer). Stable 7-days at 2-8C.

Reagent Solution #3: Reconstitute one vial of Reagent 3 with 10 mL of Reagent 4 (Enzyme Diluent). Stable 20-days at 2-8C.

PRECAUTION AND WARNING

This product is not classified as a dangerous substance. Reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

SYSTEM REQUIREMENTS

Wavelength λ = 340nm Lightpath 1 cm Temperature +37°C Reaction end point (increase) Allow reagents to reach working temperature before using.

TESTING PROCEDURE - manual

Pipet working reagent into cuvettes.

Pipette into Cuvettes	Reagent Blank	Reaction
	Cuvette	Cuvettes
Standard or Sample		100µL
DI water	100µL	
Reagent Soln. #2	2 mL	2 mL
Mix, wait 2-min., read absorbance (AINITIAL) at 340 nm		
Enz. Soln. #3	1 mL	1 mL
Mix, wait 20 minutes, and read absorbance (A _{FINAL})		

1. Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.

- 2. Add Solution #2, mix and wait 2 minutes. Zero spec using Rgt. Blank; read Std. and Sample absorbance (A1).
- 3. Add Solution #3, mix and wait 20 minutes: read A2 absorbance of Reagent Blank, Standard, and Samples.

CALCULATIONS, manual:

Calculate ΔA values and G/L: $\Delta A = A_{FINAL} - (A_{INITIAL})^* 0.677$

Subtract the ΔA of the Reagent Blank from the ΔA of each Net A= $\Delta A_{\text{SAMPLE}} - \Delta A_{\text{BLANK}}$ sample and standard:

Calculate ACE Concentration (from 0.1G/L Standard) $G/L = (0.1) X (d.f.) X Net A_{SAMPLE}$

Net A_{STANDARD}

If a calculated result is above the linear-range, dilute sample and retest, multiply this test result by the dilution factor.

SIGNIFICANCE OF MEASUREMENTS

The sensory threshold is 0.10 - 0.125 G/L. Above this level, the acetaldehyde aroma is considered a defect in table wines [Liu et.al., 2000, Int'l J. Food Science & Technology 35:49-61.] Average acetaldehyde in sherries is 0.30 G/L, and contributes to sherry character; dilute sherry prior to assay, multiply result by d.f.

APPENDIX

Automated Testing

For 'ChemWell for Wine' analysis, prepare Working Reagent as described for manual analysis. 'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range. For assistance, contact Unitech Scientific LLC Technical Support.

TRADEMARKS:

"ChemWell for Wine" and "FLEX Reagent" are Trademarks of **Unitech Scientific LLC**

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METHODOLOGY & CHEMICAL PRINCIPLES

Acetaldehyde is converted to acetic acid by AIDH (Aldehyde dehydrogenase) in presence of NAD. NADH formed in the reaction is stoichiometric to the Acetaldehyde in the sample, as measure by increasing absorbance at 340nm.