

**CITRIC ACID REAGENT** *New Formulation* (Blanking Method)

Product # **CIT<sub>nf</sub>-20** (20Tests)

Linear to 1.0G/L, longer Reagent Stability

**CIT<sub>nf</sub>-40** (40Tests)

**UV-Method** for WINE, FOOD & BEVERAGES

Reagent & Procedure changes

**INTENDED USE**

**Citric Acid FLEX-REAGENT™** is intended for measuring Citric Acid concentrations in wine.

**KIT CONTENTS**

	<b>CIT<sub>nf</sub>-20</b>	<b>CIT<sub>nf</sub>-40</b>
* <b>Sample Blank</b> (liquid) automation	20mL	2 x 20 mL
* <b>R-1 LDH/MDH</b> (liquid)	18mL	2 x 18 mL
* <b>R-2 NADH</b> (liquid)	2mL	2 x 2 mL
* <b>R-3 CL Enzyme</b> (powder)	1 vial	2 vials
* <b>R4 Buffer</b> (liquid)	1.2mL	2 x 1.2mL
* <b>Citric Std 0.8G/L</b>	2mL	2mL

**SYSTEM REQUIREMENT**

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

**SAMPLES**

If wine samples must be visually clear, filter or centrifuge turbid samples, e.g. juice, must. Degas samples containing carbon dioxide, **decolorize dark red wines using PVPP** (1G/100mL sample);

**REAGENTS, STANDARD & ASSAY PREPARATION**

Kit contents are stable through the labeled expiration date when stored at 2-8 °C. Except for **R-3 CL Enzyme** (powder), these kit components are ready to use.

**Prepare REAGENTS A & B:**

To prepare **REAGENT A**, mix 9 volumes of **R-1 LDH/MDH** (liquid) with 1 volume of **R-2 NADH** (liquid); **REAGENT A** is stable **30-days refrigerated**.

Prepare **REAGENT B** just prior to testing. Add 1 mL **R4 Buffer** (liquid) to the vial of **R-3 CL Enzyme** (powder), mix gently to dissolve. Record the **time, date & exp.** on **REAGENT B** label: **Stable for 6hr at room temperature or 3-days refrigerated**.

Refer to Appendix, **AUTOMATED TESTING**

**TESTING PROCEDURE**

Two Standards are used in this procedure. Pipet Deionized Water (DI Water) for 'Std 0', the 0.8 G/L Standard provided, and each Sample into both **Blank** & Reaction sets of Cuvettes as shown:

	<b>Sample Blank Cuvettes Standards/Samples</b>	<b>Reaction Cuvettes Standards/Samples</b>
1. <b>Std 0, Std 0.8, Sample(s)</b>	<b>25µL</b>	<b>25µL</b>
2. <b>Sample Blank</b>	<b>1000µL</b>	
3. <b>Reagent A</b>		<b>900µL</b>
4. <b>Reagent B</b>		<b>100µL</b>

Mix cuvettes, incubate 20 minutes.

Zero spectrophotometer (340 nm) with Deionized Water, Read ABS of each **Sample Blank** and Reaction Cuvette.

**Manual Calculation:**

Calculate Net ABS values by subtracting from each Reaction ABS value the corresponding **Blank** ABS value:

$$\begin{aligned} \text{Net } A_{\text{STD } 0} &= A_{\text{STD } 0} - A_{\text{STD } 0\text{-BL}} \\ \text{Net } A_{\text{STD } 0.8} &= A_{\text{STD } 0.8} - A_{\text{STD } 0.8\text{-BL}} \\ \text{Net } A_{\text{SAMPLE}} &= A_{\text{SAMPLE}} - A_{\text{SAMPLE-BL}} \end{aligned}$$

To calculate Citric Acid concentrations, first subtract the  $A_{\text{STD } 0}$  value from the  $A_{\text{STD } 0.8}$  and each  $A_{\text{SAMPLE}}$  value.

$$\text{Citric Acid G/L} = 0.8 \times \frac{\text{Net } A_{\text{SAMPLE}} - \text{Net } A_{\text{STD } 0}}{\text{Net } A_{\text{STD } 0.8} - \text{Net } A_{\text{STD } 0}}$$

Where **0.8** is the G/L Citric Standard concentration.

**Test results are linear to 1.0 G/L**; dilute high samples with DI Water, retest, and multiply the result by dilution factor.

**QUALITY CONTROL**

To monitor assay performance, test a known sample (i.e. check wine or standard with known Citric Acid concentration) in each assay. If the result obtained is within 15% of expected value, the assay is 'in control' – i.e. reagents and technique are acceptable. Factors that may affect the performance of this test include instrument function, temperature, glassware cleanliness, and pipetting inaccuracy.

**APPENDIX**

**METHODOLOGY & CHEMICAL PRINCIPLES**

The Citric acid is changed in oxalacetate and acetate by CL (citrate lyase). This reaction is aided by a secondary reduction of oxalacetate (and its decarboxylated product pyruvate) in the presence of LDH (lactate dehydrogenase) & MDH (malate dehydrogenase) by NADH. The decrease in ABS at this wavelength is proportional to the concentration of Citric acid in the tested sample.

**AUTOMATED TESTING**

**'ChemWell for Wine'**

Contact Unitech Scientific Technical Service for your **NEW** CITRIC ACID *New Formulation* test procedure.

Prepare Reagents A & B as for manual testing; place Reagents A & B, Sample Blank (liquid), and Citric Standard in CW reagent rack.

MANUFACTURED BY: **UNITECH SCIENTIFIC**  
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ChemWell for Wine automatically calculates Citric Acid results based on delta ABS values (the difference of absorbance between Reaction and Blank cuvettes.)

Contact Unitech Scientific for test protocol or assistance, if necessary.

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

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