

CITRIC ACID REAGENT 0.8 G/L Std. Concentrate

Product # **CIT-20 (20Tests)**

UV-Method for WINE, FOOD & BEVERAGES

change in Reagent & Procedure

CIT-40 (40Tests)

INTENDED USE

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

KIT CONTENTS

	CIT-20	CIT-40
* Buffer R-1	30mL	2 x 30 mL
* MDH/NADH R-2	20mL	2 x 20 mL
* CL Enzyme R-3	0.5mL	2 x 0.5mL
* Citric Standard 0.8G/L	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, LINEARITY

Kit contents are stable through the labeled expiration date when stored at 2-8°C.

The Citric Standard is supplied as a 1G/L concentrate:

- Prepare the 0.2G/L Citric Standard by diluting 1part **0.8G/L Citric Standard** + 3 parts DI Water
- Multi-level standards may be prepared from 0.1 – 0.4 G/L. Refer to 'Multipoint Standards' in APPENDIX below.

Wine results are linear to 0.4 G/L; dilute high samples and retest if necessary.

ASSAY PREPARATION

Prepare Working Reagent & Starter just prior to testing. To extend stability, aliquot remaining reagent into vials and store frozen for future analysis.

Working Reagent:

Dissolve a bottle of MDH/NADH R-2 with 20 mL of Buffer R-1 and mix gently until dissolved; avoid foaming, keep bottle closed. **Label the date on dissolved R-2.**

STABILITY: Solution is stable up to 5 days at 2-8°C and 30 days frozen at -20°C. FREEZE only ONE TIME FREEZE; **DO NOT REPEAT FREEZING**, as this reduces shelf life.

Working Starter R-3:

Just prior to use, dissolve a vial of CL Enzyme R-3 (citrate lyase) with 0.5 mL Buffer R-1 and mix gently until dissolved; avoid foaming, keep bottle closed. **Label time & date on dissolved R-3 vial.** **STABILITY:** Solution is stable up to **8 hours** at 2-8°C. and 30 days frozen at -20°C. FREEZE only ONE TIME; **DO NOT REPEAT FREEZING**, as this reduces shelf life.

Let the reagents reach the working temperature before use.

Refer to Appendix, AUTOMATED TESTING for related aliquot management.

TESTING PROCEDURE

1. Pipet water into the Reagent Blank cuvette; pipet standards and samples into respective cuvettes.
2. Pipet Working Reagent into cuvettes and incubate as shown in the table.

Pipette into cuvette:

	Blank	Standard	Sample
DI Water	25 ul	--	--
Standard	--	25 ul	--
Sample		--	25 ul
Working Reagent	1000 ul	1000 ul	1000 ul
Mix and incubate for 3 mins at 30/37°C			
Measure Initial ABS of each cuvette			
Working Starter	25ul	25ul	25ul
Mix well; then incubate for 10-15 mins until reaction is complete.			
Measure Final ABS of each cuvette			

3. Wait 3 minutes, zero spectrophotometer with Reagent Blank and read initial absorbance (A_{INITIAL}).
4. Gently mix MDH Suspension by inversion and pipet into cuvettes, mix.
5. Incubate 10-15 minutes, read Final Absorbance.

CALCULATIONS

Calculate ΔA values and G/L as follows for each cuvette:

$$\Delta A = A_{\text{FINAL}} - A_{\text{INITIAL}}$$

Subtract the ΔA for the Reagent Blank from the ΔA for each sample and standard:

$$\text{Net A} = \Delta A_{\text{SAMPLE}} - \Delta A_{\text{BLANK}}$$

Calculate Citric Acid G/L (based on CONC.)

$$\text{G/L} = 0.2 \times (\text{d.f.}) \times \frac{\text{Net A}_{\text{SAMPLE}}}{\text{Net A}_{\text{STANDARD}}}$$

Where the **Citric Standard concentration, 0.2 G/L** (i.e. following dilution from 1G/L Concentrate); **d.f.** is the wine dilution factor, if any.

QUALITY CONTROL

It is recommended to use a known 'Control' to assess assay performance. Observed recovery should be within 20% of the stated Citric Acid concentration.

APPENDIX

NOTES FOR ALTERNATE CALCULATIONS

- a. **Extinction Coefficient** (results based on factor; compare standard result with known value to verify recovery.)

Citric Acid (G/ L) = Net A X 1.281 X D.F.

$$\begin{aligned} &= \frac{\text{Net A} \times \text{MW} \times \text{T.V.}}{(\epsilon)(P)(1000\text{mG/G})(\text{SV})} \times \text{D.F.} \\ &= \frac{\text{Net A} \times 192.1 \times 1.050}{6.3 \times 1 \times 1000 \times 0.025} \end{aligned}$$

Where:

MW = 192.1 G/mole for Citric acid,

TV = total reaction volume, mL,

SV = sample volume (mL)

ϵ = absorptivity of NAD =6.3 @334-340nm; 3.4 @ 365nm

P = 1 cm light path

Recalculate if alternate WRgt & SV are used. Inaccurate SV delivery affects results; use calibrated micropipettes.

b. Multi-point standard curve Sample concentrations are calculated from the best-fit standard curve. Dilute 1G/L Citric Acid Standard with DI Water to final concentrations, e.g. 0.1, 0.2, 0.3, 0.4G/L.

METHODOLOGY & CHEMICAL PRINCIPLES

The Citric acid is changed in oxalacetate and acetate by CL (citrate lyase). This reaction is helped by a secondary one, who transforms the oxalacetate originated before (and its decarboxilated product, pyruvate) in the presence of LDH (lactate dehydrogenase), MDH (malate dehydrogenase) and NADH. The intensity of the UV-color at this wavelength is proportional to the concentration of Citric acid in the tested sample.

AUTOMATED TESTING

'ChemWell for Wine'

Wine results are linear to 0.4 G/L; contact Unitech Technical Service for guidance in adjusting your test procedure.

ChemWell automated Citric Acid uses Working Reagent and Working Starter, as prepared for manual testing. Unitech recommends preparing – and immediately freezing – appropriately sized aliquots of Working Reagent and Working Starter for future use. Refer to STABILITY claims in Assay Preparation section, Page 1. Example aliquot volumes are shown below.

Volumes for CW Testing (or freezing for future use):

	10T	35T	70T
Working Reagent Vol.	2.5 mL	8.5 mL	17mL
CW Reagent Bottle Size:	7mL	15mL	30mL
Working Starter Vol.	0.17 mL	0.30 mL	0.47mL
CW Reagent Bottle Size:	2mL		

(Volumes accounts for Reagent Bottle dead volume)

Place Working Reagent & Working Starter in CW reagent rack.

CALCULATIONS: 'ChemWell for Wine' calculates results automatically from either one standard or a multi-point standard curve; dilutes and retests values above linear range.

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

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