UNITECH SCIENTIFIC CALIFORNIA, U.S.A.

FLEX-REAGENT™

D-GLUCONIC ACID

Enzymatic, UV-Method

Product #: **GLA-F20 (20 Tests)**
GLA-F50 (50 Tests)

INTENDED USE

D-Gluconic Acid FLEX-REAGENT™ is intended for measuring D-Gluconic acid, as a marker for Botrytis in infected grape juice and must.

KIT CONTENTS	20T	50T
Sample Blank (Buffer)	20 mL	50 mL
Chromogen Diluent	18 mL	45 mL
Chromogen	2 mL	5 mL
Enzyme: GK, 6PGDH	1 mL	2.5mL
D-Gluconic Acid Std, 2.0 g/L	2 mL	2 mL

SYSTEM REQUIREMENT

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

SAMPLES

Turbid sample should be centrifuged.

REAGENTS AND STORAGE

Kit contents are stable through the labeled expiration date when stored at 2-8 °C. Reagents are liquid and ready to use.

ASSAY PREPARATION

Prepare Working Reagent

Prepare sufficient Working Reagent (W-Rgt) as shown in the table, mix gently. W-Rgt is stable 4-weeks refrigerated, bring to room temperature before use.

Working Reagent	# of Cuvettes 10	30	50
Chromogen Diluent	9	27	45
Chromogen	1	3	5

TESTING PROCEDURE

1. Two Zero-Rgt Cuvettes will be used to zero the spec. Label a Zero-Rgt Blank & Reaction Cuvette. Pipet DI Water into each of these cuvettes, as shown in table.
2. For each Standard and each Sample, label a Blank & Reaction Cuvette. Pipet each Standard & Sample into both (BL & Rx) sets of cuvettes, as shown in the table.
3. Dispense Sample Blank and W-Rgt to respective cuvettes (refer to Table) and incubate at 37C.
4. Zero the Spec with **Zero-Rgt Blank Cuvette**; read Standard & Sample Blank cuvettes.
5. Re-zero Spec with **Zero Reaction Cuvette;** read Standard and Sample Reaction cuvettes.

ADD	Volume/Cuvette: Zero-Rgt Cuvettes — Blank Cuvette	Zero-Rgt Cuvettes — Reaction Cuvette	Standard(s) & Sample(s) — Blank Cuvette	Standard(s) & Sample(s) — Reaction Cuvette
DI Water	10µL	10µL	-	-
Std or Sample			10µL	10µL
Sample Blank	1000µL	-	1000µL	-
W-Rgt	-	950µL	-	950µL
Enzyme		50µL		50µL
Mix, wait 10-15 minutes Zero Spec & **Read Blank Cuvettes** - *refer to Step #4* Zero Spec & **Read Reaction Cuvettes** - *refer to Step #5*				
Zero Spec & Read ABS	**A = 0** *"Zero* **Rgt Blank Cuvette"**	**A = 0** *"Zero* **Reaction Cuvette"**	$A_{SAMPLE-BL}$	A_{SAMPLE}

CALCULATIONS

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

Net A_{STD} = A_{STD} - A_{STD-BL}

Net A_{SAMPLE} = A_{SAMPLE} - $A_{SAMPLE-BL}$

Calculate Concentration (based on **2.0 G/L** Standard)

$$\textbf{D-Gluconic Acid G/L} = (2.0) \times (d.f.) \times \frac{\text{Net } A_{SAMPLE}}{\text{Net } A_{STANDARD}}$$

LINEARITY: For samples with initial results > 2 g/L, dilute and re-test the sample, multiply result by the dilution factor.

APPENDIX

TESTING PROCEDURE (Automation)

Contact Unitech Scientific for the ChemWell for Wine™ automated test procedures and technical support.

CHEMICAL PRINCIPLES

In presence of ATP and GluK (gluconate kinase), D-gluconic acid is phosphorylated to form D-Gluconate-6-Phosphate, which is subsequently decarboxilated by NADP in the presence of 6PGDH (6-P-gluconate-dehydrogenase). The reaction product NADPH is proportional to the concentration of D-Gluconic acid in samples, and is measured by the 340nm absorbance of the reaction.

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