

# Modified Adams Assay for Phenolics in Wine

## 1. Total Iron-Reactive Phenolics

*THIS VALUE WILL DETERMINE DILUTIONS FOR TANNIN & POLYMERIC PIGMENT ANALYSES*

- 1.1 Into a reduced volume cuvette, pipette in the following order:  
  
75  $\mu\text{L}$  of wine sample (using a 200 $\mu\text{L}$  pipette).  
800  $\mu\text{L}$  Resuspension Buffer (using repeating pipettor).  
Vortex and incubate for 10 minutes at room temperature.
- 1.2 Zero spectrophotometer with 875 $\mu\text{L}$  Resuspension Buffer at 510nm
- 1.3 Read samples at 510nm (after 10min incubation, Step 1.2).  
= **Iron-Reactive Phenolics Background.**
- 1.4 Add 125 $\mu\text{L}$  of Ferric Chloride Solution to each cuvette (using repeating pipettor).  
Vortex and incubate for 10 minutes at room temperature.
- 1.5 Add 125 $\mu\text{L}$  FeCl to zero cuvette, zero Spectrophotometer with 875 $\mu\text{L}$  resuspension buffer + 125 $\mu\text{L}$  Ferric Chloride Solution at 510nm.
- 1.6 Read samples at 510nm (after 10min incubation, Step 1.4).  
= **Iron-Reactive Phenolics Final.**

### **DISCARD ALL CUVETTES ASSOCIATED WITH THIS ANALYSIS**

- 1.7 Enter values into Total Iron-Reactive Phenolics worksheet (Wine\_Assay.xls)

**Based on the value calculated for Total Iron-Reactive Phenolics, the spreadsheet will generate dilutions for tannin and polymeric pigment analyses. Use these dilutions in parts 2 and 3 of this assay protocol.**

## 2. Polymeric Pigment - Measures "A" and "B"

Use the Wine volume and Model Wine volume generated in the Total Iron-Reactive Phenolics worksheet (Wine\_Assay.xls) in step 2.1.

2.1 Into a reduced volume cuvette, pipette in the following order:

\_\_\_\_  $\mu\text{L}$  Wine Sample - see above }  
\_\_\_\_  $\mu\text{L}$  Model Wine - see above } Total volume = 500  $\mu\text{L}$

1.0mL Washing Buffer (using repeating pipettor).

Vortex and incubate for 10 minutes at room temperature.

2.3 Zero Spectrophotometer with 1.0mL Washing Buffer at 520nm.

2.4 Read samples (Step 2.1) at 520nm.  
= **MEASUREMENT "A"**

2.5 To each cuvette add 120  $\mu\text{L}$  Bleaching Reagent (using repeating pipettor).  
Vortex and incubate for 10 minutes at room temperature.

2.6 Zero Spectrophotometer with 1.0mL Washing Buffer at 520nm.

2.7 Read samples (Step 2.5) at 520nm.  
= **MEASUREMENT "B"**

**DISCARD ALL CUVETTES ASSOCIATED WITH THIS ANALYSIS**

2.8 Enter values for **MEASUREMENT "A"** and **MEASUREMENT "B"** into the Wine Phenolics Worksheet (Wine\_Assay.xls).

### 3. Tannin & Polymeric pigment Measurement "C"

Use the Wine volume and Model Wine volume generated in the Total Iron-Reactive Phenolics worksheet (Wine\_Assay.xls) in step 3.1.

3.1 Into a 1.5mL Eppendorf tube, pipette the following:

\_\_\_\_\_  $\mu$ L Wine Sample - see above }  
\_\_\_\_\_  $\mu$ L Model Wine - see above } Total volume = 500  $\mu$ L

1.0mL Protein Solution (using repeating pipettor)

Incubate for 15 minutes at room temperature with occasional inversion

3.2 Centrifuge at maximum speed for 5 minutes to form a pellet.

#### Part I

3.3 Into a reduced volume cuvette, pipette the following:

1.0mL supernatant (from step 3.2) (using 1ml pipette)

80  $\mu$ L bleaching reagent (using repeating pipettor)

Vortex and incubate for 10 minutes at room temperature.

3.4 Zero Spectrophotometer with 1.0mL Washing Buffer at 520nm.

3.5 Read absorbance of samples (step 3.3) at 520nm  
= **MEASUREMENT "C"**.

#### DISCARD ALL CUVETTES ASSOCIATED WITH THIS ANALYSIS

3.6 Enter values for **MEASUREMENT "C"** into the Wine Phenolics Worksheet (Wine\_Assay.xls).

## Part II

- 3.7 Carefully aspirate remaining supernatant from pellet (step 3.2).
- 3.8 Add 500 $\mu$ L Washing Buffer (using repeating pipettor), close the lid and gently invert the tube.
- 3.9 Centrifuge at maximum speed for 5 minutes.
- 3.10 Carefully aspirate the supernatant.
- 3.11 Add 875 $\mu$ L of Resuspension Buffer to the pellet (step 3.9) (repeating pipettor). Incubate for 20 minutes at room temperature **WITHOUT** mixing.
- 3.12 After 20 minutes, vortex sample to resuspend pellet.
- 3.13 Transfer resuspended pellets to cuvettes (using 1mL pipette). Incubate for 10 minutes at room temperature.
- 3.14 Zero Spectrophotometer with 875 $\mu$ L Resuspension Buffer at 510nm.
- 3.15 Read samples at 510nm (Step 3.13).  
= **BACKGROUND TANNIN**
- 3.16 Add 125 $\mu$ L Ferric Chloride solution to each cuvette. Vortex and incubate for 10 minutes at room temperature.
- 3.17 Zero Spectrophotometer with 875 $\mu$ L Resuspension Buffer + 125 $\mu$ L of Ferric Chloride solution at 510nm.
- 3.18 Read absorbance of samples at 510nm (Step 3.16).  
= **FINAL TANNIN**

### **DISCARD ALL CUVETTES ASSOCIATED WITH THIS ANALYSIS**

- 3.19 Enter values for **BACKGROUND TANNIN** and **FINAL TANNIN** into the Wine Phenolics Worksheet (Wine\_Assay.xls).

## Anthocyanin, measurement "D"

4.1 Into a reduced volume cuvette, pipette in the following order:

400  $\mu\text{L}$  Model Wine (using repeating pipettor).

100  $\mu\text{L}$  wine sample (using 200  $\mu\text{L}$  pipette).

1.0mL Anthocyanin Buffer (using repeating pipettor).

Vortex and incubate for 5 minutes at room temperature.

4.2 Zero Spectrophotometer with Anthocyanin Buffer at 520nm.

4.3 Read samples at 520nm (step 4.1).

= **MEASUREMENT "D"**

**DISCARD ALL CUVETTES ASSOCIATED WITH THIS ANALYSIS**

4.4 Enter values for **MEASUREMENT "D"** into the Wine Phenolics Worksheet (Wine\_Assay.xls).

## **SOLUTION RECIPES**

### **Model Wine**

In 1.0L Schott bottle dissolve 5.0g potassium bitartrate in 800mL de-ionized (DI) water (magnetic heater/stirrer). Cool to room temperature, add 120mL of 96% Ethanol, stir 5 minutes (without heating), adjust to pH3.3 with hydrochloric acid (HCl), & make volume up 1.0L with distilled water. Store at room temperature.

### **Washing Buffer**

In 1.0L Schott bottle dissolve 9.86g sodium chloride (NaCl) in 500mL DI water, add 12mL glacial acetic acid, & adjust to pH4.9 with sodium hydroxide (NaOH). Make volume to 1.0 L with DI water. Store @ room temp.

### **Resuspension Buffer**

In 1.0L beaker, dissolve 50g SDS in 800mL of DI water, add 50mL triethanolamine, stir gently (magnetic stirrer) to dissolve SDS. When pH stabilises adjust to pH9.4 with HCl. Transfer to 1.0L Schott bottle, rinse beaker with 100mL of DI water & add to bottle. Make volume to 1.0 L with DI water. Store @ room temp.

### **Anthocyanin Buffer**

In 1.0L Schott bottle, dissolve 23g of maleic acid & 9.93g NaCl in 800mL DI water. Adjust to pH1.8 with NaOH & make to 1.0L with DI water. Store @ room temp.

### **Ferric Chloride Reagent**

In 1.0L Schott bottle, dissolve 2.7g ferric chloride in 800mL DI water, add 800 $\mu$ L conc. HCl (12.1 N; 33-37%) & make to 1.0L with DI water. Store @ room temp.

### **Bleach Solution**

In 50mL Falcon tube, dissolve 2.0g of potassium metabisulfite in 25mL DI water, prepare fresh as required. Discard unused solution.

### **Preparing Protein Stock Solution for storage**

In 500mL glass beaker, dissolve 10g of BSA (Bovine Serum Albumin) granules into 250mL of DI water to max. soluble concentration of 40mg/mL. Aliquot 1.0mL of concentrated (40mg/mL) BSA solution into screw cap vials. Store at -80°C.

### **Preparing stored stock Protein Solution for use**

Thaw frozen aliquot of protein stock solution (40mg/mL). Transfer protein stock solution to 50mL Falcon tube, add 39mL of Washing Buffer & mix well. Final concentration 1mg/mL → sufficient quantity for 40 assays.

## REAGENT LIST

Reagent Name	Supplier	Catalogue No.
SDS (sodium dodecyl sulphate) 100g	Sigma	L-5750
Triethanolamine 500mL	Sigma	T-1377
Ferric chloride hexahydrate ( $\text{FeCl}_3$ ) 100g	Sigma	F-2877
Albumin from Bovine Serum (BSA) 10g	Sigma	A3803
Sodium hydroxide Solution (NaOH) 5.0M, 150mL	Sigma	S8263
Hydrochloric acid (37% HCl) 2.5L	Sigma	435570
Glacial acetic acid 500mL	Sigma	A6283
Sodium chloride (NaCl) 500g	Sigma	S9888
Maleic Acid	Sigma	M153

## For complete analysis of each wine sample you will require:

### Solutions:

0.9mL Model Wine  
1.5mL Washing Buffer (+ 2.0mL to zero spectrophotometer)  
1.7mL Resuspension Buffer (+ 2.0mL to zero spectrophotometer)  
250 $\mu$ L Ferric Chloride Solution (+ 250 $\mu$ L to zero spectrophotometer)  
1.0mL Anthocyanin Buffer (+ 1.0mL to zero spectrophotometer)  
200 $\mu$ L Bleaching Solution  
1.0mL Protein Solution  
approx. 2.0mL of wine sample.

### Consumables:

2 x yellow (200 $\mu$ L) pipette tips.  
4 x clear/blue (1.0mL) pipette tips (2 extra tips if aspirator is not available).  
5 x reduced volume cuvettes.  
1 x 1.5ml flip-top Eppendorf tube.

### The following Combitips for the positive displacement pipette are required:

Protein = 10mL  
Model Wine = 5mL  
Washing Buffer = 10mL  
Bleaching Solution = 1.0mL  
Ferric Chloride Reagent = 2.5mL  
Anthocyanin Buffer = 10mL  
Resuspension Buffer = 10mL & 2.5mL

### Equipment:

- 1 x 200 $\mu$ L pipette
- 1 x 1000 $\mu$ L pipette (1mL)
- 1 x Eppendorf Repeater Plus
- Pipette boy (or manual equivalent) & disposable 10mL pipettes.
- Spectrophotometer
- Microcentrifuge (24 place)
- Vortex mixer
- Magnetic heater/stirrer & stir bars
- Aspirator
- pH meter & Pasteur pipettes
- Balance (3 decimal place), spatulas & weigh boats.
- 1 x 25mL measuring cylinder
- 1 x 50mL measuring cylinder
- 1 x 250mL measuring cylinder
- 1 x 1.0L glass beaker
- 6 x 1.0L Schott bottles
- 2 x 50ml Falcon tubes to make up bleach & protein solutions daily.
- 5 x 50mL Falcon tubes for day-to-day use of other solutions.
- 250 x 2.0mL screw-cap vials for storing protein solution.
- Tube Racks for 50mL Falcon tubes
- Tube racks for Eppendorf tubes