

# Measuring tannins in grapes and red wine using the MCP (methyl cellulose precipitable) tannin assay

## Scope

The MCP (methyl cellulose precipitable) tannin assay is a simple and robust means of measuring the total grape or wine tannin in red grape homogenate extracts, red wine, and other aqueous solutions (Sarneckis et al. 2005, Smith 2005, Mercurio and Smith 2006). The assay is based upon polymer-tannin interactions resulting in the formation of insoluble polymer tannin complexes which then precipitate. The polymer used is methyl cellulose, a form of polysaccharide, and therefore the assay measures the tannin that is precipitated by methyl cellulose, that is, MCP tannin. The assay is based on subtracting the absorbance values at 280 nm ( $A_{280}$ ) of solutions both with and without precipitation measured using a UV-Visible spectrophotometer. Methyl cellulose itself is non-absorbing at 280 nm, and therefore does not interfere with the assay. The method requires a control sample (i.e. no methyl cellulose added) and a treatment sample to be prepared. The  $A_{280}$  value of the control sample indicates the value for all phenolic compounds (total phenolics), whereas the  $A_{280}$  value of the treated sample indicates the value for phenolic compounds remaining in solution after the MCP tannin has precipitated. By subtracting these two values, the  $A_{280}$  of the MCP tannin in a solution can be determined and then related to epicatechin equivalents or used as an arbitrary value.

## Equipment and apparatus?

1. A calibrated spectrophotometer (traditional, plate reader, or other type) capable of measuring absorbance at 280 nm with a wavelength accuracy of  $\pm 2$  nm. Note that if using a style other than traditional, the calculation may differ from that presented here and must be validated before use.
2. Cuvettes suitable for the instrument being used (see below)
3. Centrifuge capable of a radial centrifugal force (RCF) of at least 1800g (i.e. 4,000 rpm with a 10 cm rotating radius).
4. Mixing device such as a rotary suspension mixer, shaker table or roller mixer.

The assay may be conducted in several different volume formats and the further relevant equipment requirements are described below.

### 10 mL Assay

1. 10 mm pathlength cuvettes - either quartz or acrylic disposable cuvettes with optical window below 280 nm.
2. 10 mL centrifuge tubes.
3. Pipettes for accurate dispensing of 0.25 mL to 7.75 mL volumes.
4. Centrifuge suitable for 10 mL centrifuge tubes.



### 1 mL Assay

1. 10 mm pathlength cuvettes suitable for 1 mL volume — either quartz or acrylic disposable cuvettes with optical window below 280 nm.
2. 1.5 mL centrifuge tubes.
3. Pipettes for accurate dispensing of 25  $\mu$ L to 775  $\mu$ L volumes.
4. Centrifuge suitable for 1.5 mL centrifuge tubes.

### **High Throughput (HTP) Assay (Plate Reader)**

1. 96-well deep well plates and corresponding aluminium sealing foil and silicone sealing mats (e.g. Axygen 1.1 mL) for performing the assay.
2. 96-well plates (e.g. Greiner UV Star 370  $\mu$ L) for reading absorbance.
3. Multi-channel pipette for accurate dispensing from 25  $\mu$ L to 775  $\mu$ L volumes.
4. Centrifuge and rotor suitable for deep well 96-well plates.

## Reagents

### 1. Saturated ammonium sulfate solution

To a 500mL screw closure flask (e.g. Schott bottle) add 300 mL deionised water. Add ammonium sulfate crystals (Sigma Aldrich A4915) with stirring until in excess and will no longer dissolve. Keep adding until approximately 1.5 cm of ammonium sulfate crystals are resting on the bottom of the flask. The solution is stable at room temperature for 6 months - add more ammonium sulfate if only a small amount of crystals remain in the bottom of the flask.

### 2. 0.04% methyl cellulose solution

Heat a 300 mL aliquot of deionised water to 80°C and separately cool another aliquot of approximately 700 mL to 0-5°C. Weigh 0.4g of methyl cellulose (Methyl Cellulose Sigma M-0387, viscosity of 2% aqueous solution at 20 °C equals 1,500 centipoises). To a 1 L volumetric flask add the 300 mL of hot deionised water and add small portions of the polymer powder whilst stirring quickly with a magnetic stirrer until all the polymer has been added. Avoid the formation of large gelatinous clumps by stirring at a speed such that a large vortex forms. Use a Pasteur pipette to rinse hot water down the side of the volumetric flask to rinse any polymer stuck to the neck. Add the remainder of the water (0-5°C) to lower the temperature of the solution and stir for 20-40 mins immersed in ice water (0-5°C). Remove from ice water and continue stirring until the solution becomes clear (this may have to be left stirring overnight). Allow solution to reach room temperature before making up to final volume of 1 L with deionised water. The solution is stable at room temperature for 2 weeks. Methyl cellulose may fall out of solution with time; if this does occur discard the solution.

## Procedure

Below is a general procedure for performing the MCP tannin assay. Volumes for each of the three formats (10 mL, 1 mL or HTP) are shown in Table 1.

### Control sample

1. Add the same volume of grape homogenate extract or wine as added in the treatment sample (see Table 1 for volumes).
2. Add the required volume of saturated ammonium sulfate solution (see Table 1 for volumes).

3. Make volume up to 10 mL (10 mL format) or 1 mL (1 mL or HTP format) with deionised water (see Table 1 for volumes) and mix\*.
4. Allow solution to sit for 10 minutes at room temperature.
5. Centrifuge for 5 minutes at 4,000 rpm (10 mL format), 10,000 rpm (1 mL format) or 2,000 rpm (HTP format).
6. Pipette solution into a cuvette (fill cuvette for 10 mL or 1 mL formats) or 96- well plate (300  $\mu$ L for HTP format) and record the absorbance of the solution at 280nm: **A<sub>280</sub>(control)**.

#### Treatment sample

##### **50% ethanol (non-acidified) grape homogenate extract**

1. Add required volume of methyl cellulose solution (see Table 1 for volumes) to required volume of grape homogenate extract and shake lightly several times (with foil sealer for HTP format), leave to stand for approximately 2-3 min.
2. Add required volume of saturated ammonium sulfate solution (see Table 1 for volumes).
3. Make volume up to 10 mL (10 mL format) or 1 mL (1 mL or HTP format) with deionised water (see Table 1 for volumes) and mix\*.
4. Allow solution to sit for 10 minutes at room temperature.
5. Centrifuge for 5 minutes at 4,000 rpm (10 mL format), 10,000 rpm (1 mL format) or 2,000 rpm (HTP format, sealed with silicone mat).
6. Pipette supernatant into a cuvette (fill cuvette for 10 mL or 1 mL formats) or 96-well plate (300  $\mu$ L for HTP format) and record the absorbance of the solution at 280nm: **A<sub>280</sub>(supernatant)**.

**Or**

##### **Red wine**

1. Add required volume of methyl cellulose solution (see Table 1 for volumes) to required volume of red wine and shake lightly several times (with foil sealer for HTP format), leave to stand for approximately 2-3 min. (smaller volumes of wine samples may occasionally need to be used in order to fall in the working range of the UV-Vis spectrophotometer).
2. Add required volume of saturated ammonium sulfate solution (see Table 1 for volumes).
3. Make volume up to 10 mL (10 mL format) or 1 mL (1 mL or HTP format) with deionised water (see Table 1 for volumes) and mix\*.
4. Allow solution to sit for 10 minutes at room temperature.
5. Centrifuge for 5 minutes at 4,000 rpm (10 mL format), 10,000 rpm (1 mL format) or 2,000 rpm (HTP format, sealed with silicone mat).
6. Pipette supernatant into a cuvette (fill cuvette for 10 mL or 1 mL formats) or 96-well plate (300  $\mu$ L for HTP format) and record the absorbance of the solution at 280nm: **A<sub>280</sub>(supernatant)**.

\*Once the HTP format is made up to total volume, seal with silicone mat and mix by gently shaking the plate, either by inversion several times or ideally on an automated flatbed plate shaker.

**Table 1. Optimised volumes of sample and reagents for all three formats of the MCP Tannin Assay for wine and grape extract samples.**

Sample type	Assay format	Treatment				Control			
		Sample volume	Polymer	Salt	Water	Sample volume	Polymer	Salt	Water
Wine	10 mL	0.25 mL	3 mL	2 mL	4.75 mL	0.25 mL	0 mL	2 mL	7.75 mL
	1 mL	25 µL	300 µL	200 µL	400 µL	25 µL	0 µL	200 µL	700 µL
	HTP	25 µL	300 µL	200 µL	400 µL	25 µL	0 µL	200 µL	700 µL
Extract	10 mL	1 mL	3 mL	2 mL	4 mL	1 mL	0 mL	2 mL	7 mL
	1 mL	100 µL	300 µL	200 µL	400 µL	100 µL	0 µL	200 µL	700 µL
	HTP	100 µL	300 µL	200 µL	400 µL	100 µL	0 µL	200 µL	700 µL

## Calculations

$$A_{280}(\text{tannin}) = A_{280}(\text{control}) - A_{280}(\text{supernatant})$$

$$\text{Tannin concentration (mg/L epicatechin eq.)} = {}^a \text{tannin} \times {}^b \text{DF}$$

<sup>a</sup> tannin (mg/L epicatechin eq.); calculated from the epicatechin calibration curve (see example below)

<sup>b</sup> DF (dilution factor); 40 for wine, 10 for grape homogenate extract.

### Grape homogenate extract

Tannin from grape homogenate extract can be reported as mg/L in the extract (calculated as above) or mg/g in the homogenate. The conversion to mg/g in the homogenate from mg/L in the extract is shown below;

$$\text{Tannin concentration in homogenate (mg/g)} = \frac{[\text{Tannin}]_e \times V_e}{W_h}$$

where:

$[\text{Tannin}]_e$  = tannin concentration in extract (mg/L epicatechin eq.)

$V_e$  = final volume of extract (L)

$W_h$  = initial weight of homogenate sample (g)

## Example for wine (using the AWRI epicatechin calibration)

The following information is an example only of the calibration results obtained using the AWRI's SpectraMax M2 UV-Vis plate reader spectrophotometer.

A red wine has an  $A_{280}(\text{tannin}) = 0.8$

$$\text{Tannin concentration} = \left( \frac{A_{280}(\text{tannin}) - 0.0154}{0.0124} \right) \times 40$$

$$\text{Tannin concentration} = \left( \frac{0.8 - 0.0154}{0.0124} \right) \times 40$$

Tannin concentration = 2530 mg/L

Tannin concentration = 2.53 g/L epicatechin eq.

NOTE: The epicatechin equivalent calibration curve will need to be established on the UV-Vis spectrophotometer that is to be used for the tannin assay. This can be done by volumetrically preparing epicatechin standards in water ranging in concentration from 10 to 250 mg/L and then measuring the  $A_{280}$ . The calibration curve can then be used to report results in epicatechin equivalents.

## Interpretation of results

Generally speaking, the amount of tannin in red grapes and wine will depend on the variety, regional source of the fruit, climatic conditions during the growing season and the winemaking processes used. Analysts should be aware of the variable nature of grapegrowing and winemaking and the following data (Table 2) are provided for indicative purposes only. The data are sourced from a set of analytical data for red grapes and wine from three varieties sourced from a range of regions across Australia.

**Table 2. Concentration of MCP tannin in red grapes (2006) and red wine (2004) by variety from a range of growing regions in Australia.**

	Tannin	Shiraz	Cabernet Sauvignon	Merlot
Grape extract (mg/g homogenate)*	maximum	8.0	7.86	8.35
	minimum	2.39	2.56	1.93
	average	4.15	5.50	5.35
	N	103	84	65
Red Wine (g/L)*	maximum	4.8	4.17	2.98
	minimum	0.36	0.12	0.31
	average	1.85	1.97	1.54
	N	411	325	88

Note: \* epicatechin equivalents      N = number of samples

### Estimation of uncertainty

Repeated sampling and analysis of wines and grape homogenate extracts by one operator on one plate reader and one normal spectrophotometer at the AWRI gave a range of average coefficient of variation (CV; standard deviation relative to mean) for the various assay formats as shown in Table 3. It must be stressed that the uncertainty of measurement should be determined and validated for each individual laboratory's situation using standard recognised practices that take into account all possible variables including operators and equipment used in the conduct of the assay. In practical terms, the uncertainty of measurement can often be estimated as 2 x CV (for a 95% confidence interval) as determined by the laboratory. Therefore, allowing for other variations, such as operator and daily operation, it might be reasonable to use up to 15% as an estimate of the uncertainty of measurement.

**Table 3. Average coefficient of variation (%) for the MCP Tannin assay in various formats and matrices as determined at the AWRI laboratories.**

	10 mL assay	1 mL assay	HTP (96-well)
Red wine	3.8	7.5	6.0
Red grape homogenate extract	3.0	3.2	4.4

### Quality assurance

1. Duplicate determinations should be run according to the degree of confidence required by the laboratory. This can range from as frequently as one duplicate in every five samples, or as little as one sample every week depending on the situation. Where duplicates are used, the average of the results should agree to within the defined limits of uncertainty of measurement.
2. The performance of the spectrophotometer must be checked according to standard procedures (e.g. Australian Standard AS 3753-2001), and should include as a minimum:
  - ensuring adequate instrument warm-up to avoid excessive drift; and
  - routine instrument and calibration performance checks to ensure wavelength accuracy, absorbance accuracy and repeatability.
3. Other critical equipment such as balances and volume measuring devices must be calibrated as described in standard procedures (e.g. Morris and Fen 2002; NATA 1995; AS 2162.2-1998).
4. It is recommended that an aqueous grape seed tannin solution is prepared, frozen and used as a standard for monitoring assay performance over time.

### References

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