

# ***Protein Precipitable Phenolics Microplate Assay***

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## ***Reagents***

- 1 mg/mL BSA in buffer A (as for the Protein Precipitable phenolics)
- SDS/TEA as for Protein precipitable phenolics
- FeCl<sub>3</sub> as for Protein precipitable phenolics

## ***Materials***

- 96 well plate
- Jitterbug or other device for shaking plates
- Desk top centrifuge with adaptors for 96 well plates
- Plate washer with programming
- Plate reader for visible spectrophotometric assay

## ***Method***

Use tannin at about 2.5-5.0 mg/mL dissolved in buffer, water, or methanol. samples dissolved in acetone or aqueous acetone cannot be used.

1. Set up a template for the plate, including standards, unknowns and blanks
2. In each well mix 12.5 uL BSA with 62.5 uL buffer A. Mix the plate using the *Jitterbug*.
3. Add 25 uL tannin and mix immediately with Jitterbug. If the volume of sample must be varied, add solvent to adjust sample volume to 25 uL before adding tannin.
4. Allow the plate to incubate at room temperature for 15 minutes.
5. Centrifuge at 4,000 rpm for 1 h 15 min.
6. Aspirate off the supernatant using the plate washer on an aspirate only mode. You need to set the height of the dispensers so that essentially all of the liquid is removed, without touching and removing any of the pellet.
7. Add 200 uL SDS/TEA to each well and shake plate until precipitates are completely redissolved.
8. Add 50 uL ferric chloride to each well and shake.
9. After 15 minutes read the absorbance at 510 nm.