**Chlorophyll extraction with DMSO protocol**

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This protocol explains how to use Dimethyl sulfoxide (DMSO) to extract chlorophyll (chl) from leaf tissues. The DMSO works as an organic solvent that breaks up the plasma membranes of the chloroplasts.

*Supplies needed*

Leaf tissue

Cork borer

DMSO

Glass test tubes

Oven-proof test rack

Genesys 20 Spectrophotometer

Glass curvette for spectrophotometer

10 mL graduated cylinder

Parafilm and aluminum foil

*Protocol*

1. Tissue collection
   1. Use cork borer to extract tissue
   2. Avoid the large veins (have too much structural tissue, not enough photosynthetic tissue)
   3. Get 5 discs per leaf type
   4. Measure total area collected
      1. Scan analyzed with ImageJ work well
2. Put disc(s) in 10 ml of DMSO. You must WEAR LATEX GLOVES FOR THIS!
   1. Measure the DMSO out using the graduated cylinders.
   2. Label the tubes with a sharpie! We need to know which sample is which
   3. Cover tubes with parafilm and aluminum foil
   4. Put your tubes in the rack
3. Place in oven at 65 C for 120 minutes
4. After chlorophyll is fully extracted from disc(s) to solution, extract 3 mL per curvette of each sample 3 times (10 mL of DMSO should get you 3 cuvettes full of 3 mL)
   1. Clean graduated cylinder out in between different tissues!
5. Measure absorbance using spectrophotometer at 649.1 nm for all samples
6. Measure absorbance using spectrophotometer at 665.1 nm for all samples
7. Wash out curvettes with water and ethanol
8. Repeat until finished with samples
9. Pour wastes solution into the DMSO waste bottle in the hood
10. Wash your glassware

After data collection is complete. Use the equation below from Wellburn (1994) to calculate how much Chl you have. Must divide by 10 mL because we extracted in 10 mL

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Calculate the amount of chlorophyll type in each tissue type using the above formulas. This will give you the amount of Chl per mL of solution. To convert this number to a biological meaningful number you must divide by the total area of the discs.

Table 1. Area of each leaf type

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Leaf type | Area disc 1 | Area disc 2 | Area disc 3 | Area disc 4 | Area disc 5 | Total Area |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

Table 2. The absorbance of the DMSO extracted samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Leaf type | Sample 1 at 665.1 nm | Sample 2 at 665.1 nm | Sample 3 at 665.1 nm | Sample 1 at 649.1 nm | Sample 2 at 649.1 nm | Sample 3 at 649.1 nm |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

Table 3.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Leaf type | Chl a/ml sample 1 | Chl a/ml sample 2 | Chl a/ml sample 3 | Chl b/ml sample 1 | Chl b/ml sample 2 | Chl b/ml sample 3 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

Final Numbers

Total Chl in \_\_\_\_\_\_\_\_\_\_\_\_ leaf Total Chl in \_\_\_\_\_\_\_\_\_\_\_\_\_ leaf

Using the numbers, you derived plus your classmates numbers on the board, which leaf type had the most Chl in their leaves? Which had the least? What your prediction correct?

Is there any other differences between these leaves that would cause differences in ability to extract Chl?