## Common experiments for Iron deficiency studies in Aksoy Lab

Prepared by Utku Deniz

## Total Chlorophyll Content:

1. Obtain the leaf sample, weigh, and record its weight.

For small samples:

1. Put in an Eppendorf tube.
2. Add 1ml 80% acetone and homogenize the sample with crushing sticks.

For large samples:

1. Put in a mortar.
2. Add ~10ml (depends on sample size) of 80% acetone and homogenize the sample with a pestle.
3. Store at 4°C overnight.
4. Centrifuge at 15000rpm and 4°C for 5 minutes.
5. Take the absorbance values at 470 nm (for carotenoids), 646.8nm (chlorophyll b), and 663.2nm (chlorophyll a) against 80% acetone as a blank.
6. Use the formula below to calculate chlorophyll a, chlorophyll b or the total chlorophyll amount.

$Total chl content (mg/g leaf FW)= \frac{\left((A\_{663.2} × 7.15\right)+(A\_{646.8} ×18.71)) × V}{1000 × FW}$

A663.2: Absorbance value at 663.2nm

A646.8: Absorbance value at 646.8nm

V: Volume that is used to crush leaf sample in step 3, in ml unit

FW: Leaf fresh weight that is obtained in step 1, in gram unit.

**Note:** For separate chlorophyll contents, discard the unwanted ones from the equation, eg. For the chl a content, discard the “(A646,8 x 18,71)” part from the equation.

**Note:** The unit of the calculation of $\left((A\_{663.2} × 7.15\right)+(A\_{646.8} ×18.71))$ is µg/ml. When multiplied by “V”, it becomes µg. When divided by 1000, it becomes mg. When divided by “FW”, it becomes mg/g leaf FW.

**Note:** Wait around 20-30 minutes before using the spectrophotometer for the lamps to warm up.

## FRO/FCR Activity Level:

Check the protocol by Emre Aksoy, DOI:[10.21769/BioProtoc.843](http://dx.doi.org/10.21769/BioProtoc.843).

1. Prepare assay solution, 0.1mM Fe(III)-EDTA and 0.3mM Ferrozine in distilled water. Prepare fresh and keep at dark, it is light-sensitive.
2. Gently obtain the root sample, weigh, and record its weight.
3. Put the root samples in an Eppendorf tube and add 0.8ml assay solution.
4. Incubate for around 24 hours in dark at room temperature.
5. Take the absorbance values at 562nm against the assay solution as a blank.
6. Use the formula below to calculate FRO activity.

$$FRO activity level as µM Fe(II)/g root FW/hr=\frac{\left({A\_{562}}/{28.6}\right)×V×1000}{FW×T}$$

A562: Absorbance value at 562nm

V: The assay solution volume that is root samples incubated in, in ml unit.

FW: Root fresh weight that is obtained in step 2, in gram unit.

T: The incubation time in step 4, in hours unit.

**Note:** Ferrozine and Fe (III) are quite light sensitive so be careful on those steps. Also, during the spectrophotometer measurement step, keep the samples at dark and take them out one by one.

**Note:** The unit of the calculation of $\left(A\_{562} / 28.6\right)$ is mM/ml. When multiplied by “V”, it becomes mM. When multiplied by 1000, it becomes µM. When divided by “FW”, it becomes µM/g root FW. When divided by “T”, it becomes µM/g root FW/hr.

**Note:** Wait around 20-30 minutes before using the spectrophotometer for the lamps to warm up.

**Note:** It is possible to decrease the molarity of the assay solution, and increase incubation time depending on the conditions.

Reference:

Aksoy, E. and Koiwa, H. (2013). Determination of Ferric Chelate Reductase Activity in the Arabidopsis thaliana Root. Bio-protocol 3(15): e843. DOI: [10.21769/BioProtoc.843](https://doi.org/10.21769/BioProtoc.843).

## Root Growth Measurement:

The steps can be changed due to the purpose of the experiment. Here, it is an example of 4 days of control + 7 days of stress application experiments.

1. After the seeds were grown for 4 days in control conditions, transfer the plants to stress condition and mark the tip of the roots on the surface of the plate, it can be noted as Day0.
2. After 7 days of stress application, a plate in the figure below will be obtained. Photograph or scan the plate as it can be seen in the figure, with a ruler beside it.
3. Run the ImageJ tool:
	1. Go to “File” and click open “Open…” (Ctrl+O) the file.
	2. Select the “Straight Line” and draw a 2 cm line on the ruler in the file.
	3. Go to “Analyze”, and click “Set scale…”
	4. Change the “Known distance” to 2.0 and click OK.
	5. Select the “Segmented Line”.
	6. Start from the marks of Day0, and draw the line by clicking on the root till the root tip. Right-click on to root tip to finish the drawing.
	7. Ctrl+M to record the length.
	8. Repeat steps f and g for all the samples.
	9. Copy the results from the “Results” page.



**Note:** If you need to mark the root tip day by day and measure all of them: in step f, start with left click to draw the line, on every spot you want to measure use left click instead of right-click and Ctrl+M instantly without moving the cursor. Then continue to the next spot with a left-click and repeat them for the rest. For the last one, right-click to finish the drawing.