

Extraction of oospores from leaves

Extraction of oospores from (single) leaves (small volume: 1 – 5 leaves)

- Determine surface area or weight of the leaves
- Put the leave(s) in a 50 ml blue cap (Greiner tube)
- Add 1 – 2 spoons crushed ice (\pm 5 ml) and 5 ml water of 4°C
- Homogenize sample with an Ultra Turrax mixer at 24000 rpm for 1 ½ minute. (Look out for foamy samples)
- Clean the mixer with water (siphon)
- Clean the mixer with water, alcohol (96%) and water in between samples
- Put the samples in a cold room (4°C) until the samples have reached 4°C
- Homogenize the sample again without adding water or ice for 1 ½ minute
- Add 5 mg cellulase (Duchefa cat.no. C 8001) and 5 mg macerase (Duchefa cat.no. M 8002) per sample
- Incubate the samples for 2 hours while gently shaking at room temperature
- Sonicate the samples 2 x 5 minutes
- Incubate the samples at room temperature at least overnight (best result are obtained by incubating for 2 days).
- Wash the samples on a 75 μ m and a 20 μ m sieve with running tap water
- Carry over the debris from the 20 μ m sieve to a 50 ml blue cap
- Concentrate the sample by centrifuging for 3 minutes at 5000 g and pour off liquid until 5 or 10 ml remains
- Determine the concentration of oospores with a haemocytometer (Fuchs Rosenthal)
- Dry sample containing the oospores at room temperature
- Store oospores in dry debris at room temperature

Extraction of oospores from air dried leaves (large volume:> 10 - 35 g/ dried sample)

- Determine the weight of the dry samples
- Remove the petioles from the samples (retain leaflets)
- For samples from the field remove sand by careful washing in water
- Carry over the leaves into the beaker of a kitchen blender
- Add \pm 50 ml crushed ice and 100 ml ice cold water (4°C)
- Blend the sample at low speed for 1 minute
- Wash the debris in the lit and top of the beaker down into the beaker
- Blend the sample again at high speed for ½ -1 minute
- Transfer the sample to a 500 ml Duran bottle
- Homogenize the sample with an Ultra Turrax mixer at 24000 rpm for 1 ½ - 2 minutes. (Look out for foamy samples)
- Clean the mixer with water, alcohol (96%) and water in between samples
- Put the samples in a cold room (4°C) until the samples have reached 4°C
- Homogenize the sample again without adding water or ice for 1 ½ minute
- Add 50 mg cellulase (Duchefa cat.no. C 8001) and 50 mg macerase (Duchefa cat.no. M 8002) per 10 grams of sample
- Incubate the samples for 2 hours while gently shaking at room temperature
- Sonicate the samples 2 x 5 minutes
- Incubate the samples at room temperature at least overnight (best results are obtained with incubating for 2 days).

- Wash the samples on a 250 μm , 125 μm , 75 μm and a 20 μm sieve-set with running tap water (you only need the sample on the 20 μm sieve, but because of all the tissue it's better to use the other sieves too. Sometimes it is necessary to divide the sample in portions). Pour off liquid until the required volume
- Transfer the debris on the 20 μm sieve to an 250 ml duran bottle or a large centrifuge tube
- Concentrate the sample by centrifuging for 3 minutes at 5000 g or leave the sample for 1 day to settle down.
- Determine the number of oospores with a haemocytometer (Fuchs Rosenthal)
- Dry sample with oospores at room temperature
- Store oospores in dry debris at room temperature