

Sep 06, 2019

## Flame Photometry Protocol

DOI

[dx.doi.org/10.17504/protocols.io.6t6here](https://dx.doi.org/10.17504/protocols.io.6t6here)

Flame  
Photometry

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DOI: [dx.doi.org/10.17504/protocols.io.6t6here](https://dx.doi.org/10.17504/protocols.io.6t6here)

External link: <https://s3.amazonaws.com/protocols-files/public/1f8f9bb30c61f25c81516791ba9c5a103411075f46778de9554988689ed417f6/bms2jshn.docx>

**Protocol Citation:** Mariam Awlia 2019. Flame Photometry Protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.6t6here>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** August 26, 2019

**Last Modified:** September 06, 2019

**Protocol Integer ID:** 27230

## Attachments



[bms2jshn.docx](#)

36KB

## Safety warnings

- ❗
  - Work in the fume hood.
  - Wear lab coat, eye protection, gloves and lower screen in front of face.
  - Always add acid to water slowly, never water to acid.
  - Please see SDS (Safety Data Sheet) for hazards and safety warnings.

## Preparation of 1% nitric acid



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### Safety information



- Work in the fume hood.
- Wear lab coat, eye protection, gloves and lower screen in front of face.
- Always add acid to water slowly, never water to acid.

### Note


For 250 samples (250\*5=1.25L), 1.5L of 1% nitric acid should be sufficient.

If stock is 70% nitric acid, pour  69 mL of MilliQ-water for every  1 mL of nitric acid. (x 21.4 for 1.5L).

If stock is 69% nitric acid, pour  68 mL of MilliQ-water for every  1 mL of nitric acid. (x 21.8 for 1.5L)


Pour  21.8 mL nitric acid to  1483.4 mL MilliQ-water.

## Preparation of samples

2 Put all samples in  10 mL falcon tubes and determine fresh/dry weight of plant material.

3 Add  3 mL of 1% nitric acid to  10 mL tubes of samples and close lids tightly.

4 Put in oven at  70 °C overnight.

5 After  03:00:00, shake gently without breaking the rosette to cover all in nitric acid.

6 Remove samples and let them cool down on the bench before diluting.



## Preparation of dilutions

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### Note

- Prepare dilutions in fresh MilliQ-water and make sure to be extremely precise in all measurements.
- Prepare replicates for each sample, just in case.

Use 2 salt and 2 control Eppendorfs to test the dilutions and standards before doing the rest of the samples.

Flip the falcon tube to remove condensation, before pipetting.

### Note

*Suggested dilutions:* **Salt** 1:20 (1.90ml H<sub>2</sub>O + 100µL sample)

**Control** 1:5 (1.6ml H<sub>2</sub>O + 400µL sample)

*Suggested standards:* **Salt** 500µM NaCl, 100µM KCl

**Control** 10-100µM NaCl, 1000µM KCl



### Note

Make sure to avoid carrying over any plant-fibre to the dilutions.


## Operation of flame photometer

- 8 Fill two beakers with fresh MilliQ-water; the running solution and blank solution.
- 9 Put the running solution under the U-tube and add water with droplet to the connecting tube.
- 10 Check that the U-tube doesn't have any air bubbles; wiggle it around if necessary.



- 11 Check waste container and dispose of solution down the sink.
- 12 Open “air” on the main valve and check that liquid is being drawn into the machine and that waste liquid is flowing out into waste bucket.
- 13 Check at the back of instrument that pressure is approx. 11 psi.
- 14 Open gas bottle by turning the valve all the way, then a half-turn back.
- 15 Switch flame photometer and check that the flame has been lit, and then close the hatch.
- 16 Turn computer on to record the measurements manually. Leave the running solution under the U-tube and let it run for  00:30:00 to  01:00:00 before use.

## Calibration

- 17 Shake the standard solution bottle before use, then add around  10 mL to a beaker.
  - 17.1 Set system to dual and peak only. Put in blank solution, press “Blank”, wait till “100” is flashing, then remove blank solution.
  - 17.2 Put in selected standard solution and press “Cal” for Na and K and when “100” flashes, press “Cal” again.
  - 17.3 Press “Measure” and wait till it blinks to measure **Blank** (should be  $0\pm 2$ ) and **Standard** (should be  $100\pm 2$ ).
  - 17.4 Start measuring Eppendorf samples and wipe the tube with tissue when changing solutions.

### Note

Correct measurements for **Salt** (20 - 120) and **Control** (10 - 20)

- 17.5 Run standard as test every 12-24 Samples

#### Note

When switching between standard solutions, press “Blank” for 3 seconds to restart calibration.

## Shut down and clean-up

- 18 Make sure the running solution is still under the U-tube, so the flame photometer doesn't dry out.
- 18.1 Turn gas valve off and wait until the error E61 is displayed and flame is out, then switch off and close air.  
Dispose of solution and plant material down sink with excess water.