

NexION Sample and Standard Preparation Procedure - Seed and Leaf Tissue

Greg Ziegler

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Sample Preparation for Seed/Leaf/Root/Shoot samples

1. Weigh out tissue into digestion tube (acid washed 11ml 16mmx100mm borosilicate glass)
 1. Use weighing robot for seeds between 50 and 400 milligrams
 2. For leaves, roots, and shoots:
 1. Dry tissue in coin envelopes in 50C oven for minimum of 48 hrs.
 2. Homogenize tissue (hand crushing is usually sufficient, but mortar and pestle can be used)
 3. Subsample and weigh 100mg (if possible) into test tube
 - **Note:** Hand-weighed samples should be recorded into spreadsheet in grams using the balances automated button.
 3. See tube number diagram for tube numbering (Figure 1).
2. Pre-Digestion:
 1. If average sample size is greater than 25mg:
 1. Add 2.5 ml HNO₃ soln (HNO₃ soln = 20 ppb In in conc HNO₃; 50μL of 1000 ppm Indium added to **new** 2.5 L bottle of conc HNO₃).
 2. For experiments where average sample is less than 25mg:
 1. Add 1 ml HNO₃ soln (HNO₃ soln = 20 ppb In in conc HNO₃; 50μL of 1000 ppm Indium added to **new** 2.5 L bottle of conc HNO₃).
 - **Note:** This is the most important step of sample preparation. Be certain the bottle-top dispenser is working properly (watch for air in the dispenser syringe) and make sure that each pump is delivering the correct amount to each tube. This can be checked gravimetrically if necessary.
3. Put tubes in heating blocks.
4. Let stand at room temperature for minimum of 3 hours (typically overnight).
5. Digestion: Turn on warming block, set to maximum temperature (~95C). Digest for 3 hr (until clear, with no residue on sides).
6. Turn off heating block and let cool for about 1 hr.
7. Dilute 4x with 18MΩ H₂O (UPW):
 1. Large samples experiments (average >25mg):
 1. Dilute to 10 ml (about 7.7 ml, estimate based on amount of evaporation).
 2. Small samples experiments (average <25mg):
 1. Dilute to 4 ml (about 3 ml, estimate based on amount of evaporation).
8. Mix with stirring rod block.
 - **Note:** Getting a homogeneous mixture in this step is very important. We have tested doing this by: Shaking the tubes, pipetting up and down, and letting samples heat after dilution. All of these resulted in more variability of Indium, indicating poor mixing of the acid in the final sample.
9. Transfer 1.2 mL of sample to 96-well autosampler tray and load on autosampler (2).

Standard and Control Solutions

1. Prepare standard stock solution as listed in Table 1.
2. Table 2 contains the dilutions for each of 6 standards. These are currently handled by the prepFAST autodiluter and only the 'Std Blk' and 'Std 6' need to be prepared. Table 3 contains the final standard concentrations of the 6 standards.
3. Control
 - Prepare a bulk control solution for each tissue/organism type by combining left over sample digests into a large plastic bottle
 - Depending on experiment this should either be a 500mL bottle or a large 4L bottle.
 - Except in the case of soybean and corn seeds these controls should be named as follows:
 - * Organism Tissue Date
 - Once used for an experiment it is **imperative** that the control solution not change. If more control is needed, it should be prepared in a new bottle with a new control name.
4. This tissue/organism matched control solution is placed on the autosampler in 50mL centrifuge tubes and run every 10th sample. This helps correct for drift both within an ICP-MS run and between ICP-MS runs which may occur weeks or months apart.
5. Analyze using NexION ICP-MS (Refer to Nexion ICP-MS Instrument Procedure)

Conversion of μg analyte/L solution (ppb) to mg analyte/kg sample (ppm)

The stanrd analytical procedure for seeds dilutes a weighed sample (X mg) into 10 mL of solution. This dilution is analyzed using the NexION, which gives results as ppb analyte. To convert to mg analyte/kg sample use this equation:

$$\frac{Y \mu\text{g Analyte}}{1000\text{mL Dilution 1}} \frac{10\text{mL Dilution 1}}{X \text{ mg Sample}} \frac{1\text{mg Analyte}}{1000\mu\text{g Analyte}} \frac{10^6\text{mg Sample}}{1\text{kg Sample}} =$$

$$\frac{Y \mu\text{g Analyte}}{X \text{ mg Sample}} (10) = Z \frac{\text{mg Analyte}}{\text{kg Sample}}$$

Tables and Figures

Table 1: Reagent volumes and final concentrations to make ionomics standard stock solution

Name	Symbol	Final Conc.	Dilution into 500 mL volumetric flask containing 10mL (2%) conc HNO ₃
Aluminum	Al	100 ppb	50 μ L of 1000 ppm reagent
Arsenic	As	20 ppb	10 μ L of 1000 ppm reagent
Boron	B	250 ppb	125 μ L of 1000 ppm reagent
Cadmium	Cd	2 ppb	10 μ L of 1000 ppm soln
Calcium	Ca	2 ppm	100 μ L of 10,000 ppm reagent
Cobalt	Co	2 ppb	10 μ L of 100 ppm soln
Copper	Cu	40 ppb	20 μ L of 1000 ppm reagent
Indium	In	20 ppb	10 μ L of 1000 ppm reagent
Iron	Fe	400 ppb	200 μ L of 1000 ppm reagent
Magnesium	Mg	50 ppm	2.5 mL of 10,000 ppm soln ¹
Sulfur	S	66 ppm	13,193 ppm soln, Included in Mg soln above
Manganese	Mn	500 ppb	250 μ L of 1000 ppm reagent
Molybdenum	Mo	20 ppb	10 μ L of 1000 ppm reagent
Nickel	Ni	10 ppb	5 μ L of 1000 ppm reagent
Phosphorous	P	100 ppm	5 mL of 10,000 ppm reagent
Potassium	K	150 ppm	7.50 mL of 10,000 ppm reagent
Rubidium	Rb	100 ppb	50.0 μ L of 1000 ppm reagent
Selenium	Se	40 ppb	20 μ L of 1000 ppm reagent
Sodium	Na	80 ppb	40 μ L of 1000 ppm reagent
Strontium	Sr	100 ppb	50.0 μ L of 1000 ppm reagent
Zinc	Zn	1000 ppb	500.0 μ L of 1000 ppm reagent

Table 2: Prepare each standard in a 50mL centrifuge tube containing 2.25mL HNO₃ and add UPW to 50mL mark.

	Standard stock volume	Dilution factor from std stock	Autosampler Position
Std Blank	0 mL	0.000	1
Std 1	0.250 mL	0.005	
Std 2	0.500 mL	0.010	
Std 3	1.00 mL	0.025	
Std 4	2.50 mL	0.050	
Std 5	5.00 mL	0.100	
Std 6	12.50 mL	0.250	2
Std Stk	50mL	1.000	3

Table 3: Concentration in standards (After dilution by prepFAST autodiluter), NexION ICP-MS.

Name	Std 1 (ppb)	Std 2 (ppb)	Std 3 (ppb)	Std 4 (ppb)	Std 5 (ppb)	Std 6 (ppb)
Boron	1.25	2.5	6.25	12.5	25	62.5
Sodium	0.4	0.8	2	4	8	20

¹Prepared from 50.704g MgSO₄·7H₂O / 500mL UPW

Name	Std 1 (ppb)	Std 2 (ppb)	Std 3 (ppb)	Std 4 (ppb)	Std 5 (ppb)	Std 6 (ppb)
Magnesium	250	500	1250	2500	5000	12500
Aluminum	0.5	1	2.5	5	10	25
Phosphorous	500	1000	2500	5000	10000	25000
Sulfur	329.8	659.6	1649	3298	6596	16490
Potassium	750	1500	3750	7500	15000	37500
Calcium	10	20	50	100	200	500
Manganese	2.5	5	12.5	25	50	125
Iron	2	4	10	20	40	100
Cobalt	0.01	0.02	0.05	0.1	0.2	0.5
Nickel	0.05	0.1	0.25	0.5	1	2.5
Copper	0.2	0.4	1	2	4	10
Zinc	5	10	25	50	100	250
Arsenic	0.1	0.2	0.5	1	2	5
Selenium	0.2	0.4	1	2	4	10
Rubidium	0.5	1	2.5	5	10	25
Strontium	0.5	1	2.5	5	10	25
Molybdenum	0.1	0.2	0.5	1	2	5
Cadmium	0.01	0.02	0.05	0.1	0.2	0.5

