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; 50μ L 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; 50μ L 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 he 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 $18M\omega$ 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:

$$\frac{Y\mu g \; Analyte}{1000mL \; Dilution \; 1} \frac{10mL \; Dilution \; 1}{X \; mg \; Sample} \frac{1mg \; Analyte}{1000\mu g \; Analyte} \frac{10^6mg \; Sample}{1kg \; Sample} = \\ \frac{Y\mu g \; Analyte}{XmgSample} (10) = Z \frac{mg \; Analyte}{kg \; Sample}$$

Tables and Figures

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

			Dilution into 500 mL volumetric flask containing 10mL (2%) conc
Name	Symbol	Final Conc.	HNO_3
Aluminum	Al	100 ppb	$50~\mu\mathrm{L}$ of 1000 ppm reagent
Arsenic	As	20 ppb	$10~\mu\mathrm{L}$ of 1000 ppm reagent
Boron	В	250 ppb	$125~\mu\mathrm{L}$ of 1000 ppm reagent
Cadmium	Cd	2 ppb	$10~\mu\mathrm{L}~\mathrm{of}~1000~\mathrm{ppm}~\mathrm{soln}$
Calcium	Ca	2 ppm	$100~\mu\mathrm{L}$ of $10{,}000~\mathrm{ppm}$ reagent
Cobalt	Co	2 ppb	$10~\mu\mathrm{L}~\mathrm{of}~100~\mathrm{ppm}~\mathrm{soln}$
Copper	Cu	40 ppb	$20~\mu L$ of 1000 ppm reagent
Indium	In	20 ppb	$10~\mu\mathrm{L}$ of 1000 ppm reagent
Iron	Fe	400 ppb	$200~\mu\mathrm{L}$ of 1000 ppm reagent
Magnesium	Mg	50 ppm	$2.5 \text{ mL of } 10,000 \text{ ppm soln}^1$
Sulfur	\mathbf{S}	66 ppm	13,193 ppm soln, Included in Mg soln above
Manganese	${ m Mn}$	500 ppb	$250~\mu\mathrm{L}$ of $1000~\mathrm{ppm}$ reagent
Molybdenum	Mo	20 ppb	$10~\mu l$ of $1000~\mathrm{ppm}$ reagent
Nickel	Ni	10 ppb	$5 \mu l$ 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	$50.0~\mu\mathrm{L}$ of $1000~\mathrm{ppm}$ reagent
Selenium	Se	40 ppb	$20~\mu\mathrm{L}$ of 1000 ppm reagent
Sodium	Na	80 ppb	$40~\mu\mathrm{L}$ of 1000 ppm reagent
Strontium	Sr	100 ppb	$50.0~\mu\mathrm{L}$ of 1000 ppm reagent
Zinc	Zn	1000 ppb	$500.0~\mu\mathrm{L}$ of $1000~\mathrm{ppm}$ 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	$0.250~\mathrm{mL}$	0.005	
Std 2	$0.500~\mathrm{mL}$	0.010	
Std 3	$1.00~\mathrm{mL}$	0.025	
Std 4	$2.50~\mathrm{mL}$	0.050	
Std 5	$5.00~\mathrm{mL}$	0.100	
Std 6	$12.50~\mathrm{mL}$	0.250	2
Std Stk	$50 \mathrm{mL}$	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

 $^{^{1}}$ Prepared 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

Tube	Numb	ers in	Weig	jhing l	Robot							
12	11	10	9	8	7	6	5	4	3	2	1	
13	14	15	16	17	18	19	20	21	22	23	24	
36	35	34	33	32	31	30	29	28	27	26	25	Rack 1
37	38	39	40	41	42	43	44	45	46	47	48	0.00000 25
60	59	58	57	56	55	54	53	52	51	50	49	
61	62	63	64	65	66	67	68	69	70	71	72	
84	83	82	81	80	79	78	77	76	75	74	73	
85	86	87	88	89	90	91	92	93	94	95	96	Rack 2
108	107	106	105	104	103	102	101	100	99	98	97	
109	110	111	112	113	114	115	116	117	118	119	120	
132	131	130	129	128	127	126	125	124	123	122	121	
133	134	135	136	137	138	139	140	141	142	143	144	
156	155	154	153	152	151	150	149	148	147	146	145	Rack 3
157	158	159	160	161	162	163	164	165	166	167	168	
180	179	178	177	176	175	174	173	172	171	170	169	
192	191	190	189	188	187	186	185	184	183	182	181	
193	194	195	196	197	198	199	200	201	202	203	204	
216	215	214	213	212	211	210	209	208	207	206	205	Rack4
217	218	219	220	221	222	223	224	225	226	227	228	
240	239	238	237	236	235	234	233	232	231	230	229	
241	242	243	244	245	246	247	248	249	250	251	252	
264	263	262	261	260	259	258	257	256	255	254	253	
265	266	267	268	269	270	271	272	273	274	275	276	Rack 5
288	287	286	285	284	283	282	281	280	279	278	277	

Figure 1: Tube numbers in the standard 288 test tubes.

Tube	Numb	ers v	/hen i	n Elan	ICP-	MS A	ıtosaı	mpler	Trays	3																										
-					Tray	1 /Dad	41		_	_	-	-	-		_	_	_	Tray	David 1				-	_	-		-	-		Tues	3 (Par	- 1\	_			
12	11	10	9	8	7 7	6	5	4	3	2	1		108	107	106	105	104	103	102	101	100	99	98	97	193	194	195	196	197	198	199	200	201	202	203	204
_		-	-	-		_	-	-	-	_			-	_	-	-			-	_	_		-	-		-	-	-	-	-	-	_	<u> </u>	<u> </u>	-	-
13	14	15	16	17	18	19	20	21	22	23	24	-	109	110	111	112	113	114	115	116	117	118	119	120	216	215	214	213	212	211	210	209	208	207	206	205
36	35	34	33	32	31	30	29	28	27	26	25		132	131	130	129	128	127	126	125	124	123	122	121	217	218	219	220	221	222	223	224	225	226	227	228
37	38	39	40	41	42	43	44	45	46	47	48		133	134	135	136	137	138	139	140	141	142	143	144	240	239	238	237	236	235	234	233	232	231	230	229
60	59	58	57	56	55	54	53	52	51	50	49		156	155	154	153	152	151	150	149	148	147	146	145	241	242	243	244	245	246	247	248	249	250	251	252
61	62	63	64	65	66	67	68	69	70	71	72		157	158	159	160	161	162	163	164	165	166	167	168	264	263	262	261	260	259	258	257	256	255	254	253
84	83	82	81	80	79	78	77	76	75	74	73		180	179	178	177	176	175	174	173	172	171	170	169	265	266	267	268	269	270	271	272	273	274	275	276
85	86	87	88	89	90	91	92	93	94	95	96		192	191	190	189	188	187	186	185	184	183	182	181	288	287	286	285	284	283	282	281	280	279	278	277
					Tray	4 (Pai	t 2)											Tray	5 (Part	t 2)										Tray	6 (Par	2)				
12	11	10	9	8	7	6	5	4	3	2	1		108	107	106	105	104	103	102	101	100	99	98	97	193	194	195	196	197	198	199	200	201	202	203	204
13	14	15	16	17	18	19	20	21	22	23	24		109	110	111	112	113	114	115	116	117	118	119	120	216	215	214	213	212	211	210	209	208	207	206	205
36	35	34	33	32	31	30	29	28	27	26	25		132	131	130	129	128	127	126	125	124	123	122	121	217	218	219	220	221	222	223	224	225	226	227	228
37	38	39	40	41	42	43	44	45	46	47	48		133	134	135	136	137	138	139	140	141	142	143	144	240	239	238	237	236	235	234	233	232	231	230	229
60	59	58	57	56	55	54	53	52	51	50	49		156	155	154	153	152	151	150	149	148	147	146	145	241	242	243	244	245	246	247	248	249	250	251	252
61	62	63	64	65	66	67	68	69	70	71	72		157	158	159	160	161	162	163	164	165	166	167	168	264	263	262	261	260	259	258	257	256	255	254	253
84	83	82	81	80	79	78	77	76	75	74	73		180	179	178	177	176	175	174	173	172	171	170	169	265	266	267	268	269	270	271	272	273	274	275	276
85	86	87	88	89	90	91	92	93	94	95	96		192	191	190	189	188	187	186	185	184	183	182	181	288	287	286	285	284	283	282	281	280	279	278	277

Figure 2: Tube numbers in the 96-well autosampler trays.