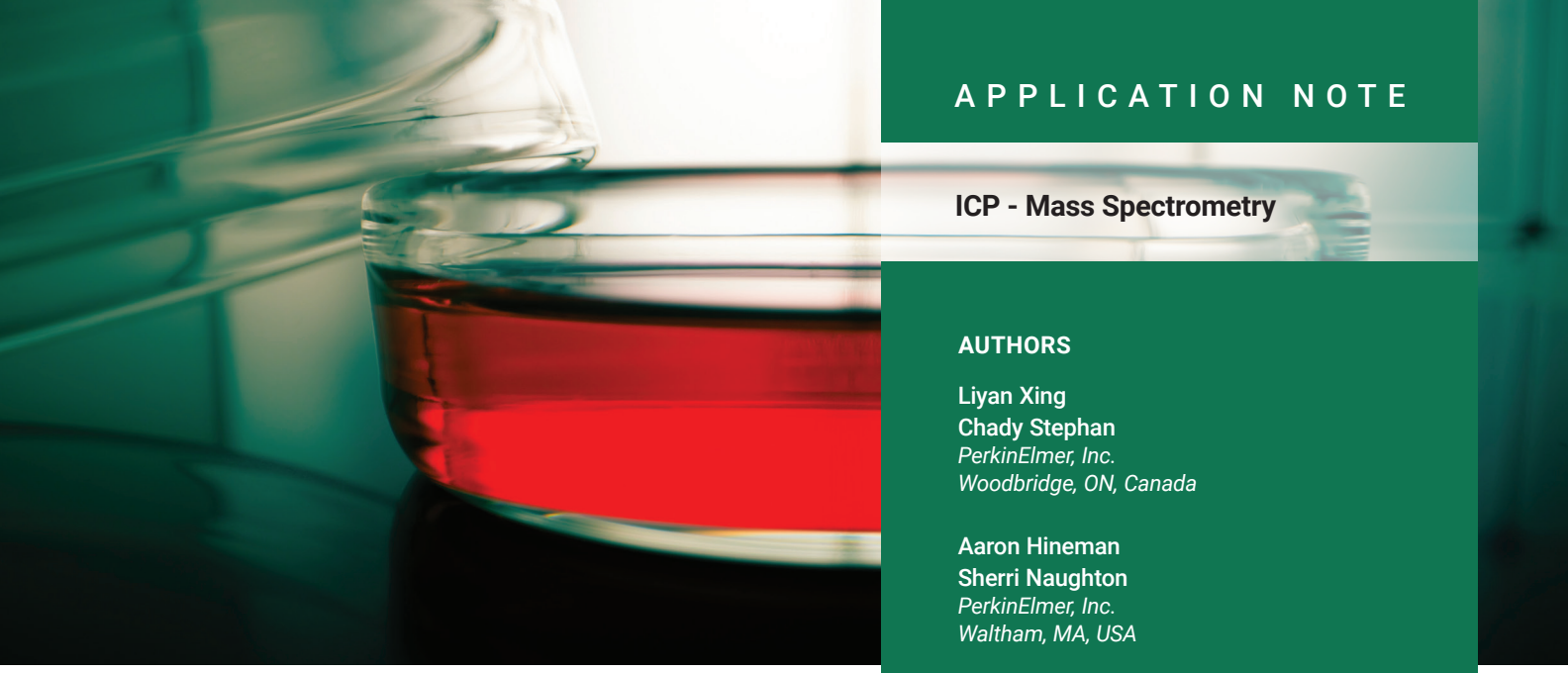

APPLICATION NOTE

ICP - Mass Spectrometry

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Direct Trace-Element Analysis in Cell Culture Media and Raw Materials with the NexION 5000 ICP-MS

Introduction

Metal ions are important components of cell culture media as they are enzyme cofactors and their variation can influence the growth of cultured cells.[1] It has been noted that the concentrations of metals in commercially available chemically-defined cell culture media can range from 1 to 25,000 ppb.[2] Raw material preparation and formulation is a known source of elemental content and variation in media; however, it is not the only contributor to trace metal concentrations and variability. Other known sources are the leaching of trace metals from bioreactors (both stainless steel and glass), preparation vessels and storage containers.[2]

This wide range of metal concentrations has a direct impact on cell growth and critical quality attributes, such as glycosylation. Therefore, source material control, trace metals determination and monitoring, as well as culture media characterization should be considered essential strategies in upstream bioprocessing.[3] Biopharma laboratories can therefore benefit from a highly sensitive and selective analytical technique, such as inductively coupled plasma-mass spectrometry (ICP-MS).

As a powerful tool for elemental analysis, ICP-MS has the advantages of multi-element analysis capability, high sensitivity, low detection limits, wide linear dynamic range, and easy automation. However, as with all other analytical techniques, ICP-MS analysis is subject to interferences. Culture media is a complex mixture containing a substantial amount of inorganic salts and organic compounds, which can generate polyatomic and other interferences that increase the spectral background and can compromise analytical accuracy.

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The NexION® 5000 Multi-Quadrupole ICP-MS is a four-quadrupole system which combines tandem mass analyzers (Q1 and Q3) with versatile Universal Cell Technology (UCT) (Q2) to achieve interference-free analysis through two basic processes: MS/MS and Mass Shift modes.[4] With MS/MS mode, analyte ions and interfering ions with the same mass are selected by Q1 and enter Q2, where the interferents react with the cell gas (e.g., NH_3, O_2, CH_4, etc.) and are ejected; examples include $^{35}Cl^{16}O^+$ vs. $^{51}V^+$, $^{39}K^{16}O^+$ vs. $^{55}Mn^+$, $^{40}Ar^{12}C^+$ and $^{40}Ca^{12}C^+$ vs. $^{52}Cr^+$, etc. With Mass Shift mode, the analyte ions react with the cell gas in Q2 to form higher mass ions that are transmitted through to Q3, and interferents are ejected; examples include $^{31}P^+ \rightarrow {}^{47}PO^+$, $^{28}Si^+ \rightarrow {}^{60}SiO_2^+$, $^{32}S^+ \rightarrow {}^{48}SO^+$, $^{75}As^+ \rightarrow {}^{91}AsO^+$, $^{78}Se^+ \rightarrow {}^{94}SeO^+$, $^{63}Cu^+ \rightarrow {}^{97}Cu(NH_3)_2^+$, etc.

The direct analysis of culture media is often plagued by high concentrations of total dissolved solids (TDS) and organic constituents, which can cause liquid path clogging, salt/carbon buildup in the interface, signal suppression or enhancement, and long-term instability. To reduce the impact of the high TDS on the sample introduction components, the PerkinElmer High Throughput System (HTS) was used to discretely introduce an optimal volume of sample into the ICP-MS system. Since it is a vacuum-driven integrated flow-injection sampling system, it also greatly improves productivity. The organic carbon content can be digested using acid(s) to decompose and oxidize the carbon which, in turn, reduces plasma loading and mitigates carbon-based interferences. For effective mineralization, the sample matrix needs to be subjected to concentrated acid and then diluted to a final volume with water for analysis. Although microwave digestion can assist in digesting the matrix and reducing carbon-based interferences, it may dilute cell-culture media below the detection limits of the instrument, increase the risk of contamination and negatively impact productivity in such settings. Therefore, having an instrument capable of directly analyzing these solutions would be highly advantageous.

Although trace elemental analysis is the theme of this application note, major elements can also provide useful information for the cell industry. Usually, major and trace elements are analyzed in separate analytical runs. The challenges associated with this approach are: 1) reduced productivity; 2) increased consumable usage and cost; and 3) increased risk of sample contamination. In order to address these challenges, an instrument capable of running both low and high concentrations within a single analytical run would be highly advantageous. The NexION 5000 ICP-MS is equipped with Extended Dynamic Range (EDR), which is able to selectively attenuate the signal, thereby overcoming the aforementioned issues.

In this application note, four commercial cell culture media samples were analyzed for both major and trace elements using the NexION 5000 Multi-Quadrupole ICP-MS equipped with HTS in a single analytical run with proper EDR settings.

Experimental

Samples and Standard Preparation

All sample and calibration solution preparation was performed volumetrically. Ultrapure water (resistivity >18.2MΩ.cm) and high-purity acids: HNO_3 (55% w/v, Tama Chemicals, Moses Lake, Washington, USA) and HCl (20% w/v, Tama Chemicals), were used for all samples, including blanks, standards, and wash solutions, unless specified otherwise. Diluted acid solutions were prepared through the dilution of the concentrated acids with ultrapure water. Isopropanol (IPA) used in internal standard and washout solutions was electronic-grade for metals (99.999% trace metals basis, Sigma-Aldrich, Oakville, Ontario, Canada). The concentrations of the diluted acid solutions were based on concentrated HNO_3 with 70% (w/v) and concentrated HCl with 37% (w/v).

Calibration Standards

Calibration standards were prepared through the dilution of commercial multi-element standards and single-element standards (PerkinElmer, Inc. - see Consumables Used table on page 6) in a diluent made of 2% HNO_3 (v/v) and 0.5% HCl (v/v) spiked with 200 µg/L gold (Au). The addition of HCl helps with the long-term stability of elements like Hg, Ag, Sn, Sb, and Mo; and gold facilitates the washout of mercury (Hg). The diluent was also used as the carrier solution, continuing calibration blank (CCB) and method blank. In order to demonstrate the accuracy of the calibration, a non-matrix matched certified reference material (CRM) was used as an initial calibration verification (ICV) standard. The concentrations of the calibration standards are shown in Table 1. Standard 5 in this standard set was used as the continuing calibration verification (CCV) standard. The CCBs and CCVs were measured periodically throughout the sample sequence.

Table 1. List of concentrations of the analytes in the calibration standards.

Analytes	Standard 1 (µg/L)	Standard 2 (µg/L)	Standard 3 (µg/L)	Standard 4 (µg/L)	Standard 5 (µg/L)	Standard 6 (µg/L)	Standard 7 (µg/L)
Li	0.1	1	10	100	200	500	1,000
Fe, Ba, Sr	0.11	1.1	11	110	220	550	1,100
Si, P, S	0.5	5	50	500	1000	2,500	5,000
Mg, K	1.01	10.1	101	1,010	2,020	5,050	10,100
Na, Ca	5.01	50.1	501	5,010	10,020	25,050	50,100
Hg	0.0002	0.002	0.02	0.2	0.4	1	2
Ag, Al, As, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Te, Th, U, V, Zn, Zr	0.01	0.1	1	10	20	50	100

Wash Solution

The wash solution consisted of 1.5% HCl (v/v) and 0.5% HNO_3 (v/v) spiked with 200 µg/L gold and 5% IPA. Isopropanol helps the washout of organic materials from the sample probe and sample loop.

Internal Standard

The internal standard solution contains 100 µg/L of Sc, 10 µg/L of Ga, and 5 µg/L each of In, Ir, Rh, and Tm, and was prepared by diluting an Internal Standard Mix solution (PerkinElmer, Inc. - see Consumables Used table on page 6) in the diluent and adding IPA (to 1%). Isopropanol was added to compensate for the matrix differences between the calibration standards and samples, and the matrix variation between samples. The internal standard was introduced into the designated port of the HTS switching valve and mixed online with the sample.

Samples

Four commercial cell culture media samples (Table 2) were analyzed.

Table 2. Cell culture media samples evaluated.

Sample Code	Sample Name	Description
I3390	Iscove's Modified Dulbecco's Medium	Liquid, sterile-filtered, with sodium bicarbonate, without L-glutamine, suitable for cell culture and hybridoma
M2279	Minimum Essential Medium Eagle	Liquid, sterile-filtered, with Earle's salts and sodium bicarbonate, without L-glutamine, suitable for cell culture
N6013	Nutrient Mixture F-10 Ham	Liquid, sterile-filtered, with sodium bicarbonate, without L-glutamine, suitable for cell culture
R8758	RPMI-1640 Medium	Liquid, sterile-filtered, with L-glutamine and sodium bicarbonate, suitable for cell culture

The working sample solutions were prepared via a 250-fold dilution of the original solution using ultrapure water. An aliquot of the diluted solution was used for the matrix spike recovery tests.

QC Samples

QC samples included the CCB, CCV, and a CRM (used as ICV). Due to the lack of cell media CRMs, a spiked sample was used in order to determine the analytical accuracy of the method.

Instrumentation

All measurements were performed using a NexION 5000 Multi-Quadrupole ICP-MS (PerkinElmer, Inc., Shelton, Connecticut, USA) equipped with HTS and an S20 series autosampler. HTS was used to minimize the total amount of sample going through the instrument as well as shorten the read delay and rinse time between samples, thus improving productivity and stability. The instrument components/parameters are shown in Table 3.

Table 3. NexION 5000 ICP-MS instrument parameters and operating conditions.

Component/Parameter	Type/Value
ICP-MS	NexION 5000
Laboratory Setting	Non-cleanroom
Nebulizer	ST-PFA MicroFlow
Spray Chamber	Quartz cyclonic
Torch	One-piece quartz torch, 2 mm injector
Interface	Pt sampler and skimmer cones Ni hyper-skimmer cone with OmniRing™ technology
Sample Uptake Rate	0.3 mL/min
RF Power	1600 W
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	1.2 L/min
Nebulizer Gas Flow	Optimized for < 2.5% oxide
Cell Gas	O_2, NH_3, He
Extended Dynamic Range (EDR)	Applied to Li, Na, Mg, S, K, Ca, Fe and Sr

Instrument Optimization

Prior to sample analysis, the instrument was tuned for optimal sensitivity and oxide as well as doubly charged ion ratios. New or newly cleaned cones need to be conditioned before optimization. In this method, the cones were conditioned by aspirating cell culture media that was leftover solution from previous runs and monitoring the internal standards until the signals were stabilized.

Results and Discussion

Method Detection Limits and Limits of Quantifications

Method detection limits (MDLs) and limits of quantifications (LOQs) were calculated as three times the standard deviation and ten times the standard deviation of ten replicated measurements of the method blank, respectively. Table 4 lists the MDLs and LOQs for the target elements. The characterization of Si, P and S is extremely important for biological matrices and is considered to be especially challenging to analyze due to the abundance of polyatomic interferences formed from the presence of ubiquitous elements such as C, N and O in air and the biological matrix. Therefore, in order to demonstrate the effectiveness of the interference removal capabilities of the NexION 5000 ICP-MS system, the MDLs of Si, P and S are highlighted in Table 4, and are much lower than what can be typically achieved with quadrupole ICP-MS systems with a single analyzer quadrupole.

Table 4. Isotopes and modes of analysis for different elements.

Element	Mass	Scan Mode	Gas Profile	MDL (µg/L)	LOQ (µg/L)
Li	7/7	MS/MS	STD	0.009	0.031
Be	9/9	MS/MS	STD	0.003	0.009
B	11/11	MS/MS	STD	0.039	0.131
Na	23/23	MS/MS	Ammonia	0.950	3.167
Mg	24/24	MS/MS	Ammonia	0.151	0.503
Al	27/27	MS/MS	Ammonia	0.012	0.040
Si	28/60	Mass Shift	Oxygen	0.520	1.733
P	31/47	Mass Shift	Oxygen	0.013	0.043
S	32/48	Mass Shift	Oxygen	0.097	0.323
K	39/39	MS/MS	Ammonia	0.121	0.405
Ca	44/60	Mass Shift	Oxygen	0.034	0.114
Ti	48/131	Mass Shift	Ammonia	0.001	0.003
V	51/51	MS/MS	Ammonia	0.0001	0.0003
Cr	52/52	MS/MS	Ammonia	0.001	0.004
Mn	55/55	MS/MS	Ammonia	0.0002	0.0008
Fe	56/56	MS/MS	Ammonia	0.005	0.017
Co	59/59	MS/MS	Ammonia	0.0004	0.001
Ni	60/111	Mass Shift	Ammonia	0.002	0.007
Cu	63/97	Mass Shift	Ammonia	0.003	0.011
Zn	66/117	Mass Shift	Ammonia	0.008	0.026
As	75/91	Mass Shift	Oxygen	0.0005	0.002
Se	78/94	Mass Shift	Oxygen	0.001	0.003
Rb	85/85	MS/MS	Ammonia	0.0002	0.001
Sr	88/104	Mass Shift	Oxygen	0.0002	0.001
Zr	90/106	Mass Shift	Oxygen	0.001	0.002
Mo	95/127	Mass Shift	Oxygen	0.001	0.002
Ag	107/141	Mass Shift	Ammonia	0.001	0.003
Cd	111/111	MS/MS	Ammonia	0.0001	0.0004
Sn	118/118	MS/MS	Ammonia	0.0005	0.002
Sb	121/121	MS/MS	Ammonia	0.0002	0.001
Ba	137/153	Mass Shift	Oxygen	0.001	0.005
Hg	202/202	MS/MS	STD	0.0003	0.001
Tl	205/205	MS/MS	STD	0.0001	0.0002
Pb	208/208	MS/MS	STD	0.0003	0.001
Th	232/232	MS/MS	STD	0.0004	0.001
U	238/238	MS/MS	STD	0.0001	0.0002

Calibration Linearity

Calibration curves were plotted after internal standard correction and blank subtraction. The coefficients of regression (R) were found to be higher than 0.9995 for all elements in the calibrated ranges. Recoveries of the calibration standards for all analytes were within ±10% in the calibration ranges.

Recovery of CRM (Acting ICV)

Each sample was measured three times over the sample run and the measurements spread randomly in the sequence. The percentage recoveries fell well within ±10% of the certified value for all certified elements in NIST 1643f (Figure 1), demonstrating the accuracy of the calibration.

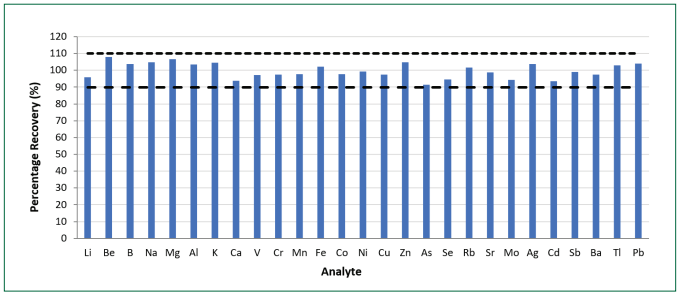

Figure 1. Recoveries for the certified elements in NIST 1643f.

Method Accuracy – Recovery of Matrix Spiked Samples

As discussed previously, selected samples (I3390, M2279 and R8758) were spiked with a multi-element solution such that the spiked concentration was the same as that of Standard 5 (Table 1) to determine the accuracy of the analytical methodology. In all cases, recoveries within ±20% were obtained for matrix spiked samples (Figure 2), thereby validating the accuracy and robustness of the analytical method.

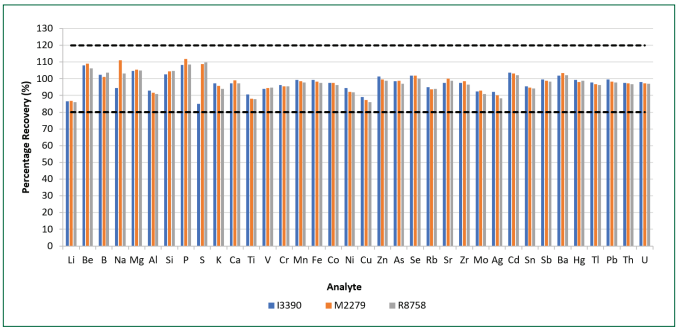

Figure 2. Recoveries for the matrix spiked standards.

Stability

To validate the long-term stability of the system for this application, four cell culture media samples were measured repeatedly over a period of 10 hours and the internal standard and CCV recoveries were monitored over this period.

Internal Standard Recovery

All the internal standard recoveries (normalized to the calibration blank) were well within ± 20% of the initial value and did not show apparent drifting throughout the sequence (Figure 3). These findings demonstrate the outstanding stability and robustness of this system over the entire 10-hour period and its suitability for extended sample runs.

CCV Recovery

All the CCV recoveries were normalized to Standard 5 (Table 1) and were found to be within ± 20% of the original reading. As shown in Figure 4, there was no apparent trending throughout the sequence, demonstrating the validity of the calibration over the 10-hour sample run. This feature is important for high-throughput laboratories in terms of overall efficiency and productivity, preventing frequent reruns of the calibration standards and reducing instrument maintenance. Consequently, it can facilitate real-time monitoring of samples, which is highly desired for quality control in a cell culture manufacturing line.

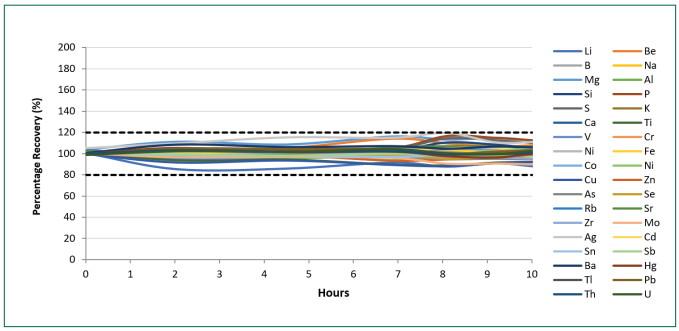

Figure 4. CCV recoveries over a 10-hour sequence of analysis of varied cell culture media samples.

Conclusions

Quality control in cell culture media is essential for reducing variability and ensuring improved production yields. In this application note, a wide spectrum of elements in cell culture media samples were analyzed with high accuracy and precision using the NexION 5000 Multi-Quadrupole ICP-MS. Here, EDR allowed for the analysis of both low and high concentrations of elements within a single analytical run which, coupled with HTS, dramatically contributed to the overall accuracy, stability, and improved productivity of the system. The award-winning NexION 5000, the industry's first four-quadrupole ICP-MS system, delivers outstanding interference removal capabilities, allowing interference-free analysis that in turn leads to extremely low detection limits. The accuracy of the method was validated with excellent recoveries of the spiked samples. Furthermore, excellent stability of the internal standards and CCV were achieved, which demonstrates the overall robustness and stability of the methodology and instrumentation.

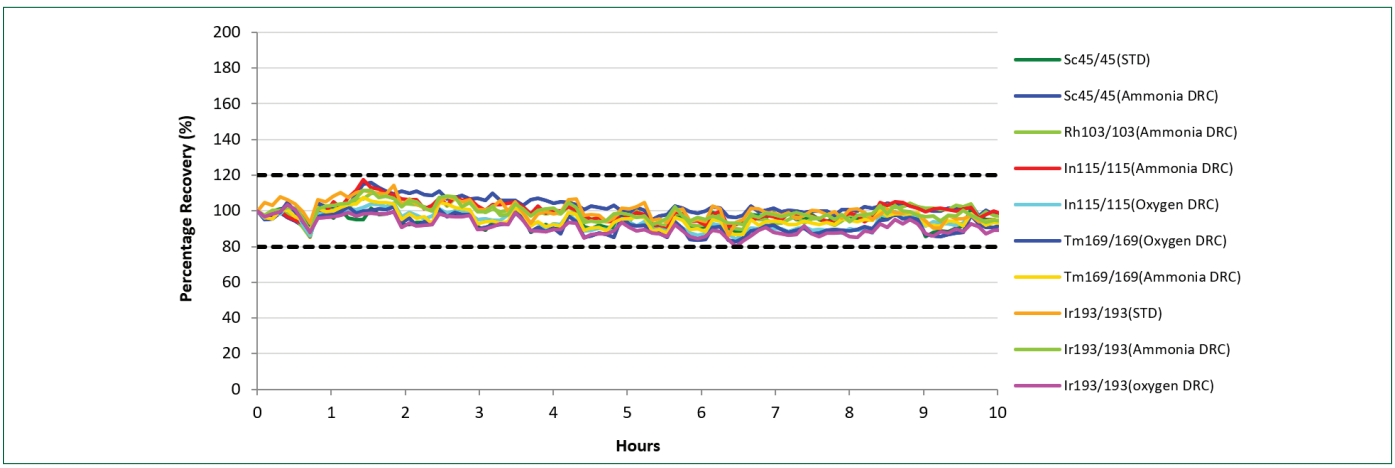

Figure 3. Internal standard recoveries during a 10-hour sequence of analysis of varied cell culture media samples.

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3. Graham R.J., Ketcham S., Mohammad A., Bandaranayake B.M.B., Cao T., Ghosh B., Weaver J., Yoon S., Faustino P.J., Ashraf M., Cruz C., Madhavarao C. Zinc supplementation improves the harvest purity of B-glucuronidase from CHO cell culture by suppressing apoptosis. *Applied Microbiology and Biotechnology,* 104 (2020) 1097-1108.
4. NexION 5000 ICP-MS Brochure, PerkinElmer, 2020.

Consumables Used

Component	Description	Part Number
Peristaltic Pump Tubing	Carrier: Green/Orange (0.38 mm i.d.) Internal Standard: Orange/Red (0.19 mm i.d.) Waste: Gray/Gray Santoprene (1.30 mm i.d.)	N8152403 N8152401 N8152415
Internal Standard Mix	200 mg/L Sc, 20 mg/L Ga, 10 mg/L In, Ir, Rh, Tm	N9307738
Multi-element Standards	100 µg/mL of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn in 5% HNO_3/trace HF	N9301721
	10 µg/mL of B, Th, U in 2% HNO_3	N9307807
Single-element Standards	1000 µg/mL of Au in 10% HCl	N9303759
	1000 µg/mL of Ba in 2% HNO_3	N9303729
	1000 µg/mL of Bi in 10% HNO_3	N9303731
	1000 µg/mL of Ca in 2% HNO_3	N9303733
	1000 µg/mL of Fe in 2% HNO_3	N9303738
	1000 µg/mL of K in 2% HNOv	N9303742
	1000 µg/mL of Li in 2% HNO_3	N9303781
	1000 µg/mL of Mg in 2% HNO_3	N9303743
	1000 µg/mL of Na in 2% HNO_3	N9303746
	1000 µg/mL of P in water	N9303788
	1000 µg/mL of Rb in 2% HNO_3	N9303792
	1000 µg/mL of S in water	N9303796
	1000 µg/mL of Si in water/0.4% HF	N9303799
	1000 µg/mL of Sr in 2% HNO_3	N9303802
	1000 µg/mL of Hg in 10% HNO_3	N9303740
	1000 µg/mL of Zr in 2% HNO_3	N9303812

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