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Intracellular iron measurements by ICP-MS

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes intracellular iron measurements performed by Inductively coupled Plasma-Mass spectrometry.



Cell lysis

- 1 Cells are pelleted by centrifugation  800 x g, 20°C for  00:05:00
- 2 Supernatant is removed and cell pellet lysed by adding  250 µL of RIPA buffer + 1% Protease and phosphatase inhibitors.
- 3 Protein concentration was measured by Pierce BCA assay

Inductively Coupled Plasma-Mass Spectrometer

- 4 Cell lysis is combined with  0.5 mL of trace metal-grade concentrated HNO₃ (67–69% w/w)
- 5 Samples are heated at  50 °C for  01:00:00
- 6 Sample digests are transferred into trace metal-grade 15ml centrifuge tubes and diluted with  4.45 mL of purified water from a Milli-Q system
- 7 Samples are spiked with  50 µL of 5µg L⁻¹ gallium
- 8 Iron concentrations are determined using a Perkin Elmer NexION 5000 Inductively Coupled Plasma-Mass Spectrometer operating in dynamic reaction cell mode with ammonia gas.
- 9 Normalise iron concentrations to total protein.