MASSACHUSETTS GENERAL HOSPITAL
HARVARD MEDICAL SCHOOL

Center for Skeletal Research
MGH Endocrine Unit

Cell Signaling & Subcellular Imaging Core
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IP_3 ^{3}H-Myo-inositol (cell lysates)

This service allows for the direct quantification of inositol phosphates (IP_1, IP_2 and IP_3) in cell lysates by using the radiometric, ^{3}H-myo-inositol-based assay that has been widely used to assess signaling via the Gαq/PLC/IP_3/$_i$Ca/PKC pathway, and typically referred to as the "Berridge assay" (*Berridge et al., Biochem. J. 1983. 212: 473-482*). Typically, cells would be cultured in 24-well plates and pre-labeled with ^{3}H-myo-inositol (2 mcCi/ml) in inositol-free-DMEM/0.1%BSA (provided by the Core) for 16 hours prior to treatment. The cells would then be treated with test drugs or control reagents in buffer containing 30 mM LiCl for 30 minutes, and the reactions terminated by adding 5% TCA. The acid lysates are then processed for extraction of IPs by anion-exchange chromatography, and the eluted IP-specific fractions (IP_1, IP_2 and IP_3) are analyzed by liquid scintillation counting. Users would provide the cells, either before or after labeling and ligand treatment, as arranged with Core personnel. Costs are calculated based on reagents and supplies on a per-sample basis.

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