

IP1

Method:	Homogenous, Time-Resolved Fluorescence (HTRF)
Vendor:	Cisbio Bioassays, France. #62IPAPEB
Description:	<p>Levels of myo-Inositol 1 phosphate (IP1) in lysates of cultured cell are measured using an Homogeneous, Time-Resolved-Fluorescence (HTRF) assay kit from Cisbio. IP1 is a metabolite in the Gαq/PLC/IP3/iCa/PKC signaling cascade and is stable in the presence of LiCl (present in the kit lysate buffer). Levels of IP1 thus reflect the activation status of this pathway. The Cisbio IP-One Tb assay is based on a FRET response between a labeled (d2) IP1 analog and a labeled (Lumi4-Tb-cryptate) monoclonal antibody specific for IP1; the unlabeled IP1 in the lysate inhibits this response by competing for the antibody binding site. The ratio of the fluorescence signals (665 nm/620 nm) from the acceptor (d2) and donor (Lumi4- Tb-cryptate) moieties provides the signal readout. Use of a fluorescence ratio minimizes possible photophysical interference from buffer conditions and colored compounds. The kit is amenable for HTS applications. Typically, cells would be cultured in 96-well plates, treated with test drugs or control reagents for 30 minutes, then lysed with the assay lysis buffer (provided by Core). The plate can then be transferred to the Core for assay completion. The Core would thus add the donor and acceptor reagents, and process the samples for data acquisition and reduction using the PerkinElmer Envision plate reader and accompanying software. User costs are based on the cost of the kit and the fraction of the kit that is consumed by the user samples (wells).</p>

Sample and Performance Characteristics

Plate /cells:	Black , Flat-Bottom 96-well plates; cells must be Intact and living, prepared per the protocol above.
Volume:	Cells in 100ul of culture medium/buffer per well.
range:	1.0-11,000 nM
Notes:	Contact Core to arrange sample submission and assay.

1

Director: Thomas Gardella, Ph.D.
 Braden Corbin, Technician
 Email: gardella@helix.mgh.harvard.edu Website:
<https://csr.mgh.harvard.edu/cell-signaling>