POSTER for XVI Spanish Drug Discovery Network Meeting 2024

Title:

High performance TR-FRET assays for the measurement of phosphorylated human STAT1, STAT3 and STAT6 in cell lysates using THUNDER® TR-FRET

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Abstract:

The Signal Transducer and Activator of Transcription (STAT) family of proteins plays a complex and essential role in mediating cellular responses to cytokines and growth factors. The abnormal activation of STAT proteins, including STAT1, STAT3 and STAT6, has been associated with malignant transformation. Therefore, the ability to assess the phosphorylation of STAT1, STAT3 and STAT6 in a cellular setting is important for drug discovery research. The homogeneous immunoassay formats available to detect and quantify these STAT proteins in cell lysates are expensive, thereby precluding its wide adoption in academic and small industrial laboratories. Here we describe the validation of improved immunoassays for the measurement in a 384-well format of phosphorylated STAT1 (Y701), STAT3 (Y705) and STAT6 (Y641) in cell lysates using the THUNDER® TR-FRET platform. These optimized cell-based assays were applied for determining the pharmacological profile of known activators and inhibitors of the JAK/STAT pathway. All assays exhibited wide dynamic ranges, high signal-to-background-ratios and Z' values higher than 0.8. Overall, the results presented here demonstrate that the novel THUNDER phospho-STAT1, phospho-STAT3 and phospho-STAT6 TR-FRET assays generate robust, reproducible data and are amenable to high-throughput screening (HTS) applications. This suite of homogeneous assays provides new tools for the screening and characterization of specific and selective modulators of the JAK/STAT signaling pathway. The THUNDER TR-FRET platform represents a cost-effective alternative to traditional homogeneous immunoassays, both in an academic setting and in HTS laboratories.