Use cell-based ERK2 LinkLight™ Assay for Identifying Molecules Modulating Cell-surface Receptors and ERK2 Activity

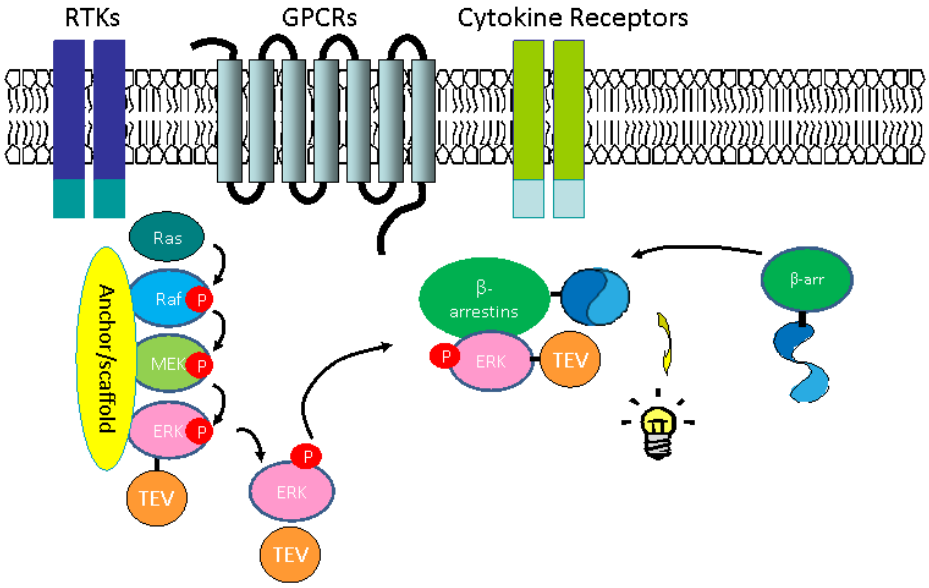
Extracellular signal-regulated kinase 2 (ERK2) is a member of the MAP kinase family and is activated in response to various extracellular stimuli. Activation of a diverse type of cell-surface receptors transmits extracellular stimuli through phosphorylation of non-receptor kinases, such as ERK2. Thus, ERK2 acts as a converging point for multiple biochemical signals, and are involved a large variety of cellular processes such as cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription[1].

Signal adaptor proteins such as β-arrestins (including β-arrestin-1 and β-arrestin-2) have no intrinsic enzymatic activity but link with other proteins to form signal transduction complexes. They are ubiquitously expressed in various types of tissues and serve as hubs to interact with a large number of proteins. The interaction partners include kinases, phosphatases, scaffold proteins, transcription factors, cytoskeletal proteins, and membrane proteins including GPCRs, RTKs, and ion channels[2,3]. The interactions facilitate the formation of large signal complexes that coordinate responses of multiple signaling pathways to incoming stimuli.

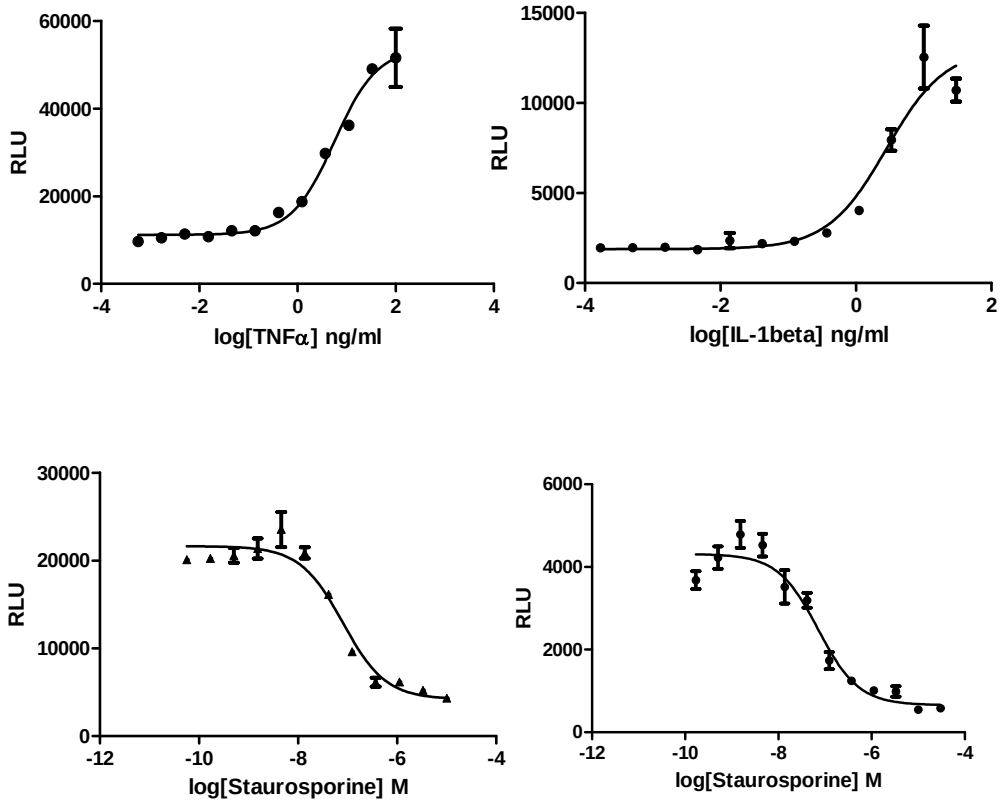
Cellular signal transductions involve highly coordinated protein-protein interactions that are regulated by protein phosphorylation and dephosphorylation. Activation of ERK2 promotes formation of ERK2/ β-arrestin signal complex. Thus, we developed cell-based LinkLight assays to assess ERK2 activity through ERK2 interaction with β-arrestins. We demonstrated that activation of diverse cell surface receptors including receptor tyrosine kinases, cytokine receptors, and G-protein coupled receptors by their corresponding ligands produced sensitive ERK2 and β-arrestin interaction signals. Specific antagonists, inhibitors as well as pan-kinase inhibitors can block the interaction signals.

In addition for assessing small molecules, LinkLight ERK2/ β-arrestin assay can be used for identification of functional antibodies against cell-surface receptors. Unlike traditional methods such as Biacore and ELISA for antibody binding affinity assays, LinkLight assay can be used to identify agonistic and antagonistic antibodies. Functional antibodies are of great interests for therapeutic drug development.

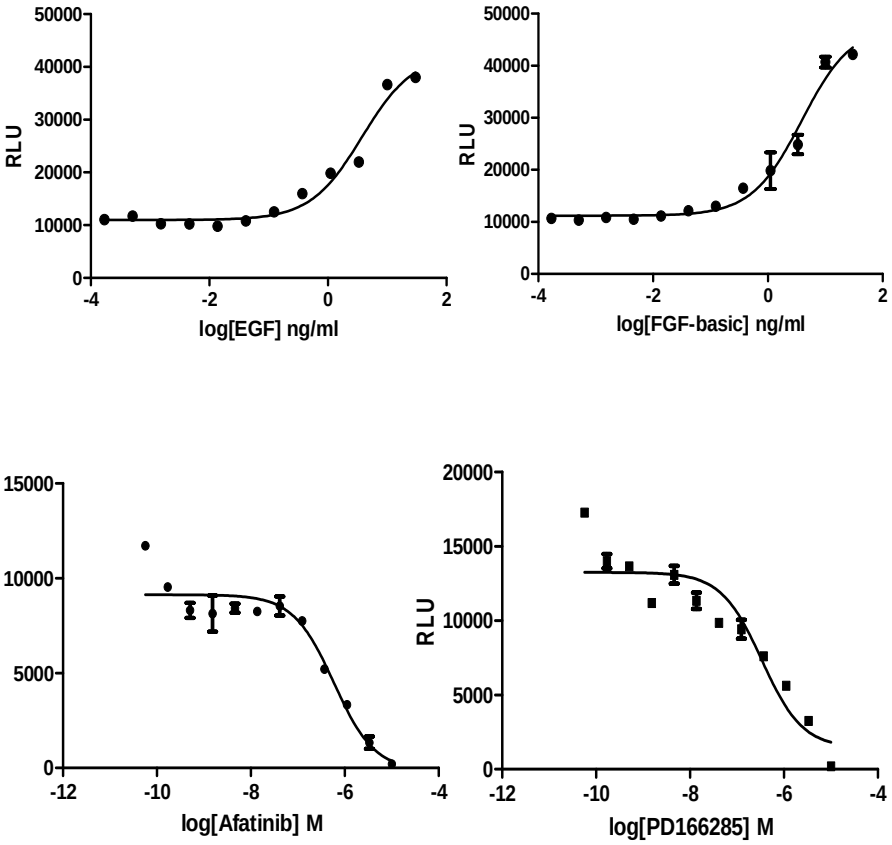
LinkLight technology[4-5] utilizes protein-protein interactions to assess cellular signaling pathways. Upon protein interactions, a bioactive luciferase is generated and luminescent signals can be sensitively detected. The signal is stable even the interaction partners are separated. The assay does not involve transcription and translation process and thus eliminates potential off-target effects. In addition, the assay is HTS-ready and detection reagents are cost-effective.



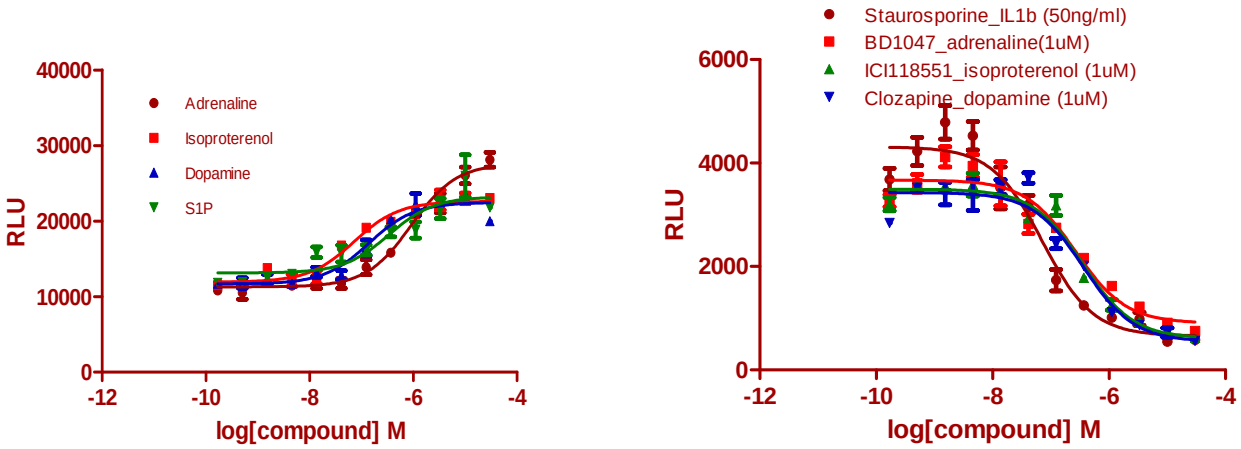
Activation of endogenous cytokine receptors such as TNFR1 and IL1R in U2OS cells produced ERK/ β-arrestin interaction signals which were blocked by a pan-kinase inhibitor staurosporine.



Activation of endogenous receptor tyrosine kinases (RTK) in U2OS cells such as EGFR and FGFR produced ERK/ β-arrestin interaction signals which were blocked by their specific inhibitors.



Activation of endogenous GPCRs in U2OS cells produced ERK/ β-arrestin interaction signals which were blocked by their specific inhibitors.



Potentially, by exogenous expression of your interested receptors in LinkLight ERK/ β-arrestin cells, you could screen and identify small molecules and biologics that modulate receptor activities.

Assay Procedure
1. Seed 10,000 to 15,000 cells per well with 40 μL culture media in a white 384-well plate, culture cells in a humidified 37°C, 5% CO2 incubator for over 16 hours.
2. Gently replace with 20 μL culture media without FBS and antibiotics.
3. Add 5 μL of serial dilution of FBS or your stimulant to wells, incubate 60~90 minutes in a humidified 37°C, 5% CO2 incubator. For antagonist assay, replace with 15 μL culture media without FBS, add 5 μL of your testing compounds and incubate for 15 min, then add 5 μL of stimulant, incubate for another 60~90 min.
4. After incubation, take the plate out and equalize to room temperature for 10 to 15 min.
5. Add 25 μL detection reagent ONE-Glo, Bright-Glo, NeoLite, or BrightLite reagent to each well, read luminescent light on an ultra-sensitive luminescence plate reader. Alternatively, you can dump off the media, add 15 μL detection reagent, and record the luminescence.

If you have any questions, please contact your sales representatives or

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References:
1. Yoon S and Seger R (2006) The extracellular signal-regulated kinase: Multiple substrates regulate diverse cellular functions. Growth Factors 24: 21-44.
2. DeWire SM, Ahn S, Lefkowitz RJ and Shenoy SK (2007) Beta-arrestins and cell signaling. Annu Rev Physiol 69: 483-510.
3. Lin A and DeFea KA (2013) beta-Arrestin-kinase scaffolds: turn them on or turn them off? Wires Syst Biol Med 5: 231-241.
4. Eishingdrelo H, Cai J, Weissensee P, Sharma P, Tocci MJ, Wright PS (2011). A cell-based protein-protein interaction method using a permuted luciferase reporter. Curr Chem Genomics 5: 122-128.
4. Eishingdrelo H, et al. (2014) ERK and β-arrestin interaction: a converging-point of signaling pathways for multiple types of cell-surface receptors. J. Biomolecular Screen (DOI: 10.1177/1087057114557233).