LinkLight ASK1 Assays

Apoptosis Signal-Regulating Kinase 1 (ASK1) is a serine/threonine kinase that is activated in response to various stress signals. ASK1 is activated in response to oxidative stress, ER stress, calcium overload, and inflammatory signals, including those induced by tumor necrosis factor alpha (TNFα) and LPS through the phosphorylation of specific serine and/or threonine residues of target proteins. ASK1 activation has been associated with diabetes, cancer, cardiovascular and neurodegenerative diseases[1].

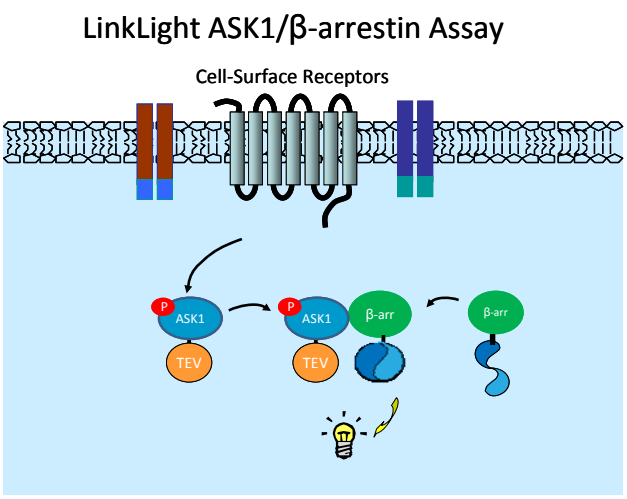
Signal adaptor proteins such as14-3-3 and β-arrestins have no intrinsic enzymatic but link with other proteins to form signal transduction complexes. They are ubiquitously expressed in various types of tissues and serve as hubs to interact with a large number of proteins. The interaction partners include 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. The interaction with adaptor proteins also contribute redistribution/re-localization of interaction partners, allowing signal transduction between different cellular compartments.

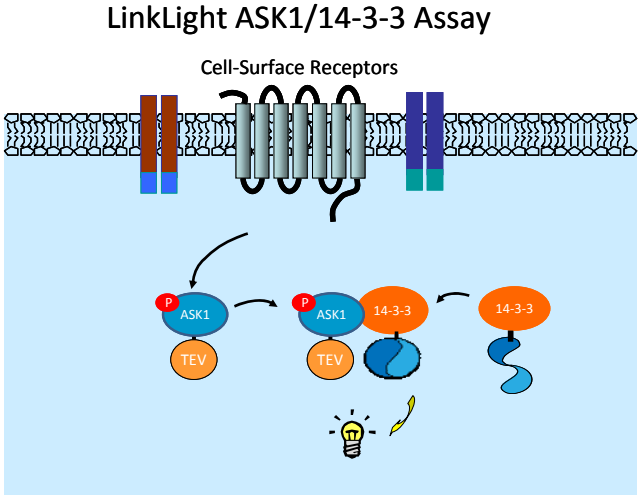
Molecules that modulate ASK1 activity and signaling pathways are of great interests for therapeutic drug development[4]. Discovery of ASK1 inhibitors is done traditionally via kinase enzymatic activity or protein phosphorylation assays, mostly in cell-free systems. However, many kinase inhibitors are identified from those assays have paradox behaves in cells. We have shown that RAF inhibitor GW5074 and MEK inhibitor CI1040 did not block down stream ERK kinase activity in our LinkLight ERK2/b-arrestin-2 interaction assay, while in combination, they inhibited ERK2 activity (manuscript submitted). The results suggest that cell-based functional assays may be more physiological relevant for identification and validation of kinase inhibitors.

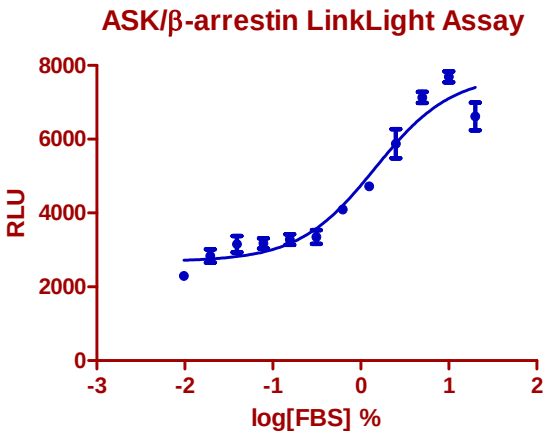
Cellular signal transductions involve highly coordinated protein-protein interactions that are regulated by protein phosphorylation and dephosphorylation. Thus, we developed cell-based assays to assess ASK1 activity through ASK1 interaction with β-arrestin-1 and 14-3-3 proteins. β-arrestin and 14-3-3 proteins are mediators in ASK1 signaling pathways.

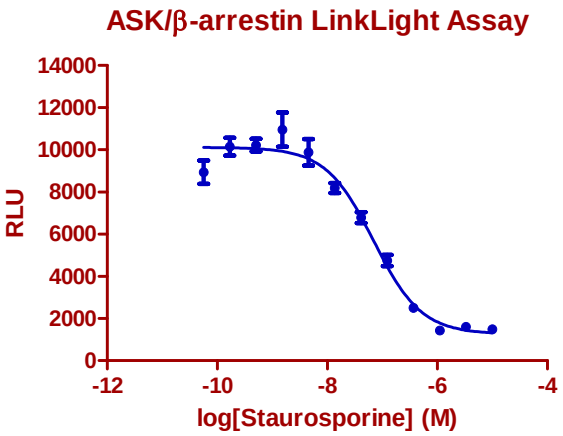
LinkLight technology[5] utilizes cell-based protein-protein interactions to assess signaling pathways. Upon protein interactions, a bioactive luciferase is generated and luminescent signals can be sensitively detected. The signal is stable even the interaction partners are separated. The assay does not 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.

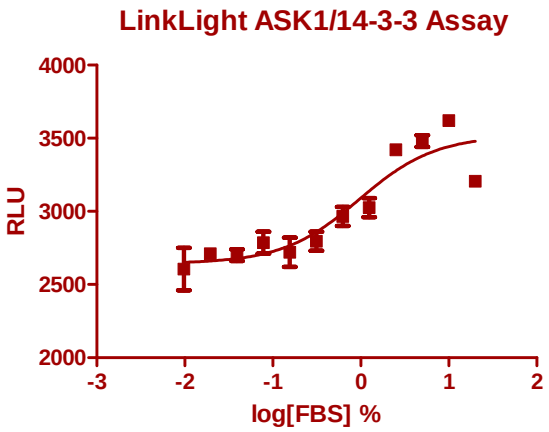
We have demonstrated fetal bovine serum treatment promoted ASK1 interaction with β-arrestin and 14-3-3, the interaction signals were blocked by a pan-kinase inhibitor staurosporine. FBS contains a number of growth factors, hormones, and ligands. Many of these factors and hormones bind and activate cell-surface receptors. The activated receptors transduce extracellular stimuli through phosphorylation of non-receptor kinases, such as ASK1. TNFα also promoted ASK1 and 14-3-3 interaction.

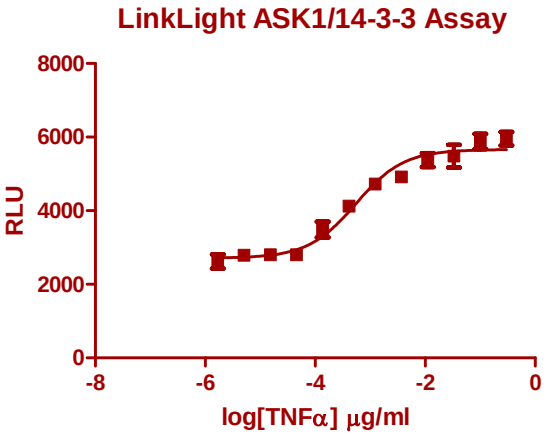

LinkLight ASK1/β-arrestin Assay
Cell-Surface Receptors
P
ASK1
TEV
β-arr


LinkLight ASK1/14-3-3 Assay
Cell-Surface Receptors
P
ASK1
TEV
14-3-3


ASK/β-arrestin LinkLight Assay
RLU
log[FBS] %


ASK/β-arrestin LinkLight Assay
RLU
log[Staurosporine] (M)


LinkLight ASK1/14-3-3 Assay
RLU
log[FBS] %


LinkLight ASK1/14-3-3 Assay
RLU
log[TNFα] µg/ml

Assay Cell Line Description
LinkLight ASK1/ β-arrestin-2/CHO cell line; catalogue number: 5006-S
LinkLight ASK1/ β-arrestin-1/U2OS cell line; catalogue number: 5027-S
LinkLight ASK1/ 14-3-3/U2OS cell line; catalogue number: 5011-S

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™ reagent (Promega, Cat. No. E6110, E61120, E61130) 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

BioInvenu Corporation,
50 Williams Parkway, Unit A-2,
East Hanover, NJ. 07936
973-585-6777
info@bioinvenu.com

References:
1. Hattori K, Naguro I, Runchel C, Ichijo H (2009). The roles of ASK family proteins in stress responses and diseases. Cell Commun. Signal 7: 9.
2. DeWire SM, Ahn S, Lefkowitz RJ and Shenoy SK (2007) Beta-arrestins and cell signaling. Annu Rev Physiol 69: 483-510.
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4. Ryoichi Hayakawa, Teruyuki Hayakawa, Kohsuke Takeda, and Hidenori Ichijo (2013). Therapeutic targets in the ASK1-dependent stress signaling pathways. Proc. Jpn. Acad., Ser. B 88: 434-453.
5. 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.