



The PathHunter assay monitors the interaction of two proteins in a whole cell, homogeneous assay format using enzyme fragment complementation. The cells have been engineered to express two complementing fragments of  $\beta$ -Gal within different cellular compartments. In this system, a small 42 AA enzyme fragment, ProLink is appended to the protein of interest such as FOXO-3. The larger enzyme fragment EA (Enzyme Acceptor) resides in the nucleus. Activation of the protein initiates translocation to the nucleus as in the FOXO-3 that forces complementation of the two enzyme fragments. This action results in the formation of fully complemented  $\beta$ -galactosidase enzyme, the activity of which is measured using chemiluminescent PathHunter Detection Reagents.