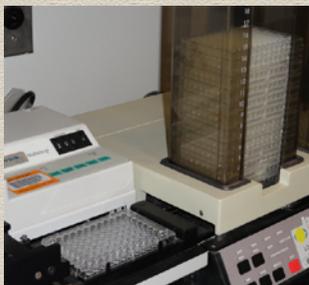




# ClariCELL™ MLK3/MKK4 Kinase Assay Service

## 4-Step Assay Validation

### Description



The **ClariCELL™ MLK3/MKK4 Kinase Assay** quantifies MLK3 (MAP3K11) dependent phosphorylation of a human full-length physiological substrate, kinase deficient (KD) MKK4 (MAP2K4), in human cells. The assay is useful to determine potencies of small-molecule inhibitors against the specified kinase in the context of a cellular environment. Compound testing services are available utilizing the assay.

### Overview

Human Embryonic Kidney (HEK 293) cells transiently expressing sequence verified human full-length MLK3 and KD MKK4 are exposed to test compound or control, then lysed to release cellular proteins. KD MKK4 is captured onto an assay plate, and the extent of substrate phosphorylation is quantified by ELISA using an antibody specific for the phosphorylation event. Cells expressing substrate alone (KD MKK4) are also utilized to validate the assay for MLK3 dependent kinase activity.

### MLK3/MKK4 Expression in Cells



Figure 1: KD MKK4 [K131M] was expressed transiently in 293 cells with or without MLK3. Following cell lysis, a Western was performed with an appropriate antibody to detect total MLK3 protein.

### MLK3/MKK4 Autophosphorylation in Cells

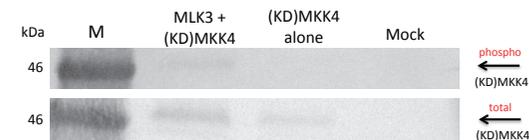


Figure 2: KD MKK4 [K131M] was expressed transiently in 293 cells with or without MLK3. Following cell lysis, an IP Western was performed with appropriate antibodies to capture and detect total and phospho-MKK4 protein.

### Quantification of Phosphorylation

#### ELISA Activity

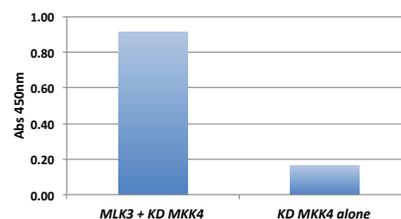


Figure 3: KD MKK4 [K131M] was expressed transiently in 293 cells with or without MLK3. Following cell lysis, an ELISA was performed to quantify the extent of phosphorylation of KD MKK4.

### Reference Inhibitor Data

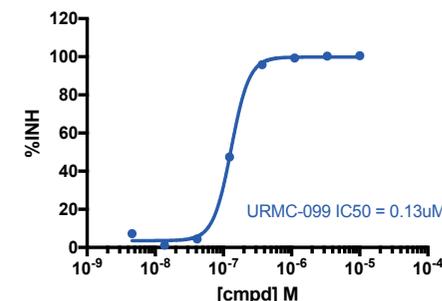


Figure 4: A phosphorylation assay was performed in the presence of URM-099, an MLK3 inhibitor. % inhibition data were plotted to determine the IC50.

To learn more, please contact

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