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Kinase assay to aid in development of therapeutics for acute myeloid leukemia

Peptide substrates to sensitively and specifically measure the real-time activity of FLT3, a kinase linked to acute myeloid leukemia.

Technology No. 20160394

IP Status: Issued US Patent; **Application #:** 15/603,273

Applications

- Clinical diagnostic to determine AML behavior and optimal treatment strategy
- Oncology research on mechanism of resistance development in cancers (specifically AML)
- Chemotherapeutic drug identification and development
- Basic kinase research

Key Benefits & Differentiators

- **Highly selective and specific:** Perform 200% better compared to the currently available FLT3 substrate, capable of providing real-time activity measurements in live cells.
- **Multiple readouts amenable to high throughput assays:** Phosphorylation of the substrate can be measured using ELISA, mass spectrometry, or through incorporation of terbium binding segments eliciting a fluorescent readout.
- **Use with wild-type, unmodified kinases:** Does not require the modification or tagging of kinases prior to experimentation.

FLT3’s heavy role in AML

Fms-like tyrosine kinase 3 (FLT3) kinase is one of the most frequently mutated genes in acute myeloid leukemia (AML). Early studies have shown that mutations in FLT3 are correlated with poor long-term prognosis with increased risk for relapse. To assess FLT3 activity, Laurie Parker’s lab at the University of Minnesota designed substrates phosphorylated by FLT3 in a manner that is highly efficient and specific. Coupled with fluorescent and/or mass spectrometric readouts, these peptides are valuable tools in both drug discovery and

diagnostics for FLT3-linked AML.

Measuring FLT3 activity to develop diagnostics and discover therapeutics

The developed FLT3 artificial substrates (FAS-A) is compatible with living cells and facilitates rapid detection of kinase activity in real time. When compared to alternatives, FAS-A is phosphorylated 200% more effectively than the only other FLT3 substrate reported in the literature (FLT3tide). Due to its high selectivity, FAS-A can be used in complex lysates or mixtures and with negligible off-target effects and can be multiplexed with other assays. Finally, the phosphorylation of the substrate can be measured using ELISA, mass spectrometry, or through incorporation of terbium binding segments, facilitating a fluorescent readout. This flexibility facilitates the use of the substrate in a wide variety of applications including high-throughput and drug discovery methods.

Phase of Development

Multiple designed substrates of wildtype and mutant forms of FLT3 assessed in vitro.

Researchers

Laurie Parker, PhD
Associate Professor, Biochemistry, Molecular Biology and Biophysics
External Link (cbs.umn.edu)

Publications

High-throughput Identification of FLT3 Wild-type and Mutant Kinase Substrate Preferences and Application to Design of Sensitive In Vitro Kinase Assay Substrates
Molecular and Cellular Proteomics, 2019

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