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LF502 NanoScan FLT Multimode Fluorescence Microplate Reader

Histon Deacetylase Activity Assay Using FLT and Nano-FP read-out

Histone deacetylases are important and currently investigated target classes in oncology. Selective lead structures are required to improve efficacy and reduce adverse effects. Common assays used so far to identify new lead structures are affected by many false positive hits due to auto-fluorescence of compounds or triggering undesired signal transduction pathways. These drawbacks are diminished by a dual parameter competition assay using the red fluorescent label Atto 700. The assay is intended to monitor the enzymatic activity of zinc-dependent Histon deacetylases. A new fluorescent inhibitor probe shows an increase in both, **fluorescence anisotropy (Nano-FP)** and **fluorescence lifetime (FLT)** upon binding to the enzyme. The assay is well suited for high-throughput screening.

Materials:

Microplate reader:	LF502 NanoScan FLT
Software:	Mikrowin 2000
Enzyme:	HDAH (histone deacetylase-like amidohydrolase)
Ligand:	Atto700-AH (Atto700-aminohexanoyl-hydroxamate)
Microplates:	384 well black microplates (Greiner)

Method:

The assay buffer consisted of 250 mM sodium chloride, 15 mM Tris–HCl, and 20 mM potassium chloride including 0.001% Pluronic F-127 at pH 8.0. The Atto700-HA conjugate (see Fig. 2A) was bound by HDAH in assay buffer resulting in the increase of fluorescence anisotropy and fluorescence lifetime.

Fluorescence lifetime was measured with excitation at 633 nm and detection at 680/30 nm within a 50-ns detection window. For the measurement of fluorescence polarization values excitation occurred at 635 nm with vertically polarized light while the fluorescence was split up into a parallel polarized ($I_{//}$) and a vertical polarized (I_{\perp}) fraction. Both fractions were measured simultaneously with two photomultipliers by integrating the corresponding time resolved fluorescence decay curve between 4 and 20 ns.

Further NanoScan Settings:

Laser frequency:	30 Hz
Average over no. of laser pulses:	16
Laser energy:	10 of 100
Gain Channel 1:	reduced gain
Integration window for Nano-FP:	4 – 20 ns

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Dose-response curve of an inhibitor:

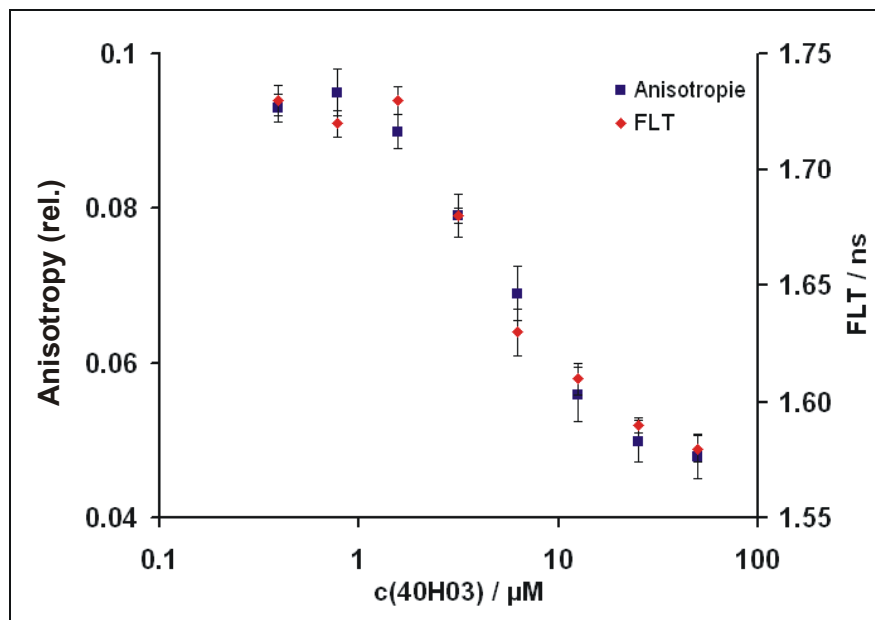


Figure 3: Dose-response curves of the inhibitor 4OH03 with HADH. Both, fluorescence anisotropy and fluorescence lifetime, are plotted versus the inhibitor concentration.

Summary:

The described binding assay is characterized by excellent performance in both signal readouts fluorescence anisotropy and fluorescence lifetime. The assay procedure is extremely simple and involves only the addition of preformed complex consisting of Atto700-HA and HDAH to screening compounds or controls, equilibrating and measuring. One of the strengths of the presented assay is its robustness against autofluorescent compounds. The LF502 NanoScan supports both read-out methods thus providing two highly robust and internally comparable data sets.

Acknowledgement:

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Reference:

Riester D, Hildmann C, Haus P, Galetovic A, Schober A, Schwienhorst A, Meyer-Almes FJ. Non-isotopic dual parameter competition assay suitable for high-throughput screening of histone deacetylases. *Bioorg Med Chem Lett*. 2009 Jul 1;19(13):3651-6. Epub 2009 May 3.