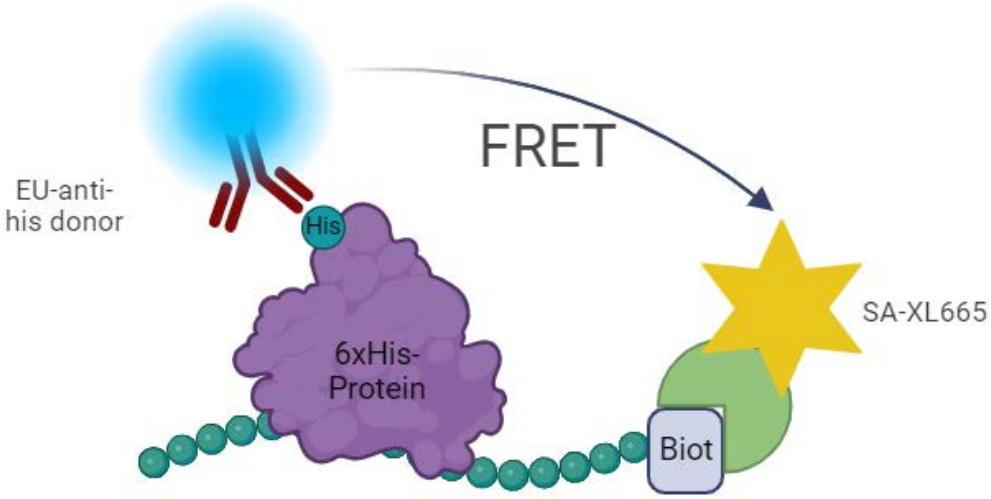
HTRF assay for YEATS domain peptide interaction

1. Rationale/Aim

This procedure is intended to measure the interaction between YEATS domain proteins and histone peptides modified by an appropriate epigenetic mark and can be used to assay compounds which bind to the YEATS domain and inhibit this interaction. The assay uses 6His tagged YEATS domain proteins with an anti-6His antibody conjugated with Eu3+ chelate (donor) and a biotinylated peptide with Streptavidin conjugated XL-665 (acceptor). When the donor and acceptor labels are in close proximity, by binding of the peptide by the YEATS domain, the excitation of the donor (337 nm) triggers a Fluorescence Resonance Energy Transfer (FRET) to the acceptor which then fluoresces at specific wavelength (665 nm).



2. Experimental conditions

2.1 Key Requirement:

- *Echo acoustic liquid handler (Labcyte, Beckman Coulter, USA).*
- *Plate reader using the HTRF module with dual emission protocol (channel A = ex. 320nm, em. 665nm and channel B = ex. 320nm, em. 620nm).*
- *Multidrop™ Combi Reagent Dispenser (ThermoFisher Scientific, USA).*
- *ProxiPlate-384 Plus, White 384-shallow well Microplate (PerkinElmer, USA).*
- *Assay buffer: 25mM HEPES pH 7.0, 20mM NaCl, 0.05% Tween-20, 0.05% BSA.*
- *Excel (Microsoft, USA)and GraphPad Prism (Dotmatics, USA) softwares*

2.2 Key Resources Table:

A list of key materials (in table format). Must include vendors and Cat. No. For in-house materials, provide internal IDs.

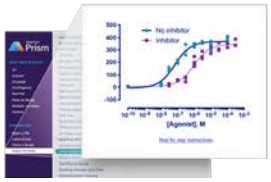
Reagents (items)	*Suppliers*	*Cat. No.*
LANCE Eu-W1024 Anti-6xHis	*Cisbio/PErkinElmer*	AD0111
Streptavidin-XL665	*CisBio*	610SAXLF
Recombinant His-tag Protein of interest	*In-house*	*none*#*
Biotinylated peptide	*LifeTein*	*Custom**

** The concentrations of His-tagged protein and biotinylated peptide used in the reaction have to be determined beforehand by HRTF protein and peptide titration in order to get the highest non-saturating signal.*

 # Custom expression and purification with >80% purity.

3. Protocol

3.1 General workflow

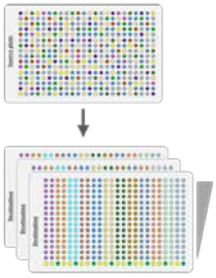


Dispense inhibitors to be tested onto 384-well plate
Include DMSO and positive controls

Prepare 2x protein-peptide mix and dispense 10 µL per well.
Incubate 1 hour at room temperature
Prepare 2x HTRF reagent mix and dispense 10 µL per well.
Incubate 1 hour at room temperature

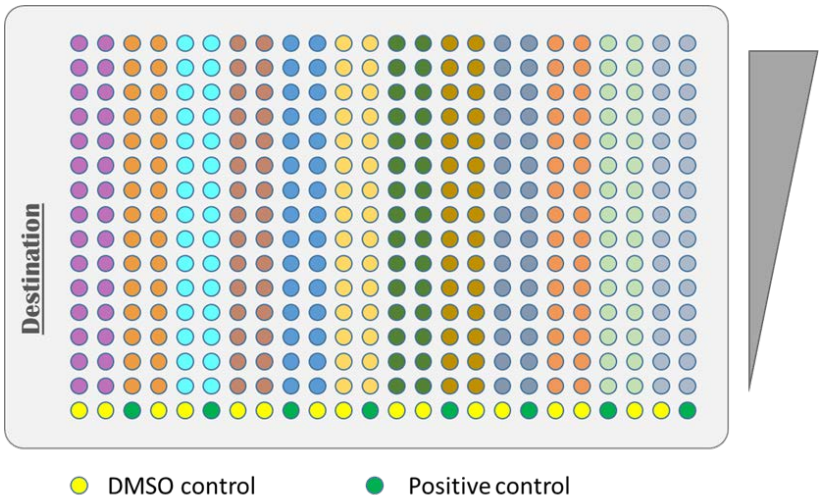
Read the plate fluorescence using the appropriate HTRF module

Analyse the data using a non-linear fit curve (non-linear four parameter) to determine IC50



3.2 Workflow

The compounds to be tested were dissolved in DMSO at a stock concentration of 10 mM and dispensed into microplate using the Echo acoustic liquid handler. The assay covers a window of compound concentrations from 20 µM to 20 nM spread in 15 data points in duplicate. Solvent and inhibitor controls were added to each plate in order to calculate the background signal.



The 2X mix of 6xHis-protein (MLLT1A-His, 3.12 nM) and biotinylated peptide (H3K9cro-Biot, 12.5nM) was prepared in assay buffer (20mM NaCl, 25mM HEPES pH 7.0, 0.05% BSA and 0.05% Tween-20)and 10 µl were added to the dispensed compounds and incubated for one hour at room temperature. The 2X detection mix was prepared by mixing SA-APC (at 1/4000 dilution) and anti-His-Eu (at 1/40,000 dilution) and 10 µl were added to each well. The plate was incubated for another hour at room temperature in the dark. The plate was read in order to calculate the HTRF specific signal and the % inhibition values were calculated as follows:

% Inhibition = (positive control-signal)/(positive control) ×100

The IC50 values were determined by plotting % inhibition vs compound concentration and fitting the data to a non-linear sigmoidal dose response equation (4 parameter fit):

$$I_i = I_o + \frac{I_{complete} - I_o}{1 + \left(\frac{IC_{50}}{c_i}\right)^s}$$

I_i: Inhibition at given concentration c_i

$I_{complete}$: Complete absence of binding, bottom of curve

I_0: No inhibition of binding, top of curve

IC_{50}: Concentration at which inhibition is 50%

c_i: concentration i in range tested

s: Hill coefficient (slope at IC50)

Calculations were carried out in Excel and GraphPad Prism templates.