EPIgeneous™ Methyltransferase Assay

ELRIG Drug Discovery Meeting

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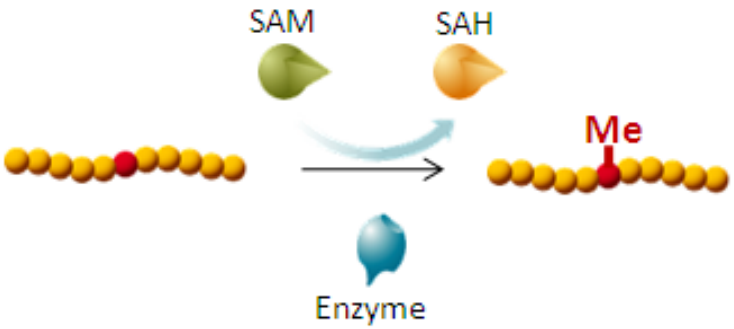
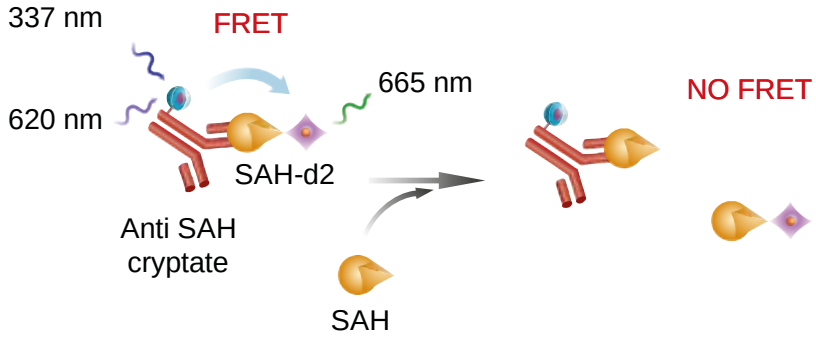
EPIgeneous™ Methyltransferase Assay

An adaptable assay for substrates, enzymes, inhibitors and SAM concentrations

- Assay principle
  - Competition assay
  - The methyltransferase activity is assessed through SAM to SAH conversion.
  - A direct measurement of SAH is done with an anti SAH antibody.



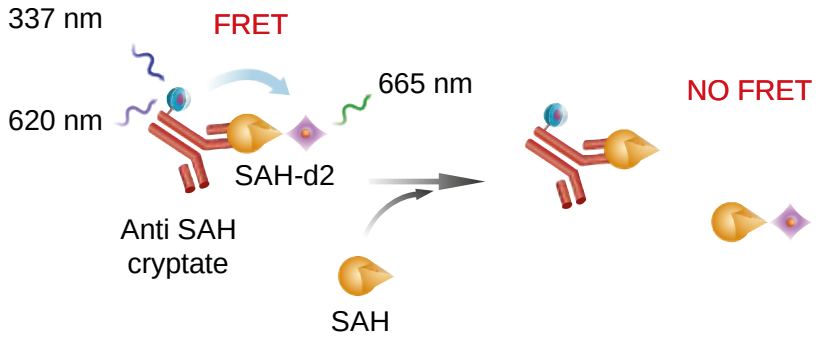


EPIgeneous™ Methyltransferase Assay

An adaptable methyltransferase assay

- Assay principle
  - Based on HTRF® Technology

HTRF® principle: just mix & measure



EPIgeneous™ Methyltransferase Assay

HTRF principle

Spectral selectivity

337 nm

665 nm

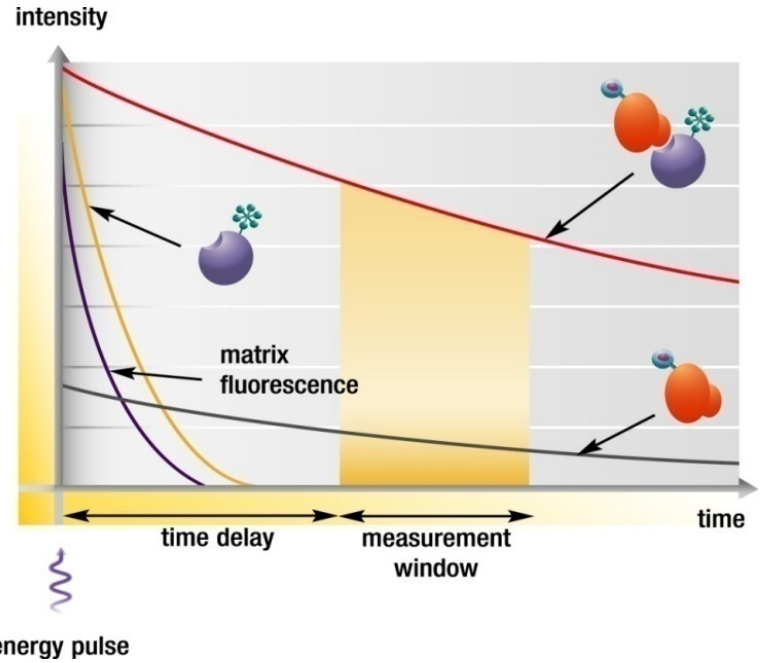
Donor

long-life fluorescence (ms)

Acceptor

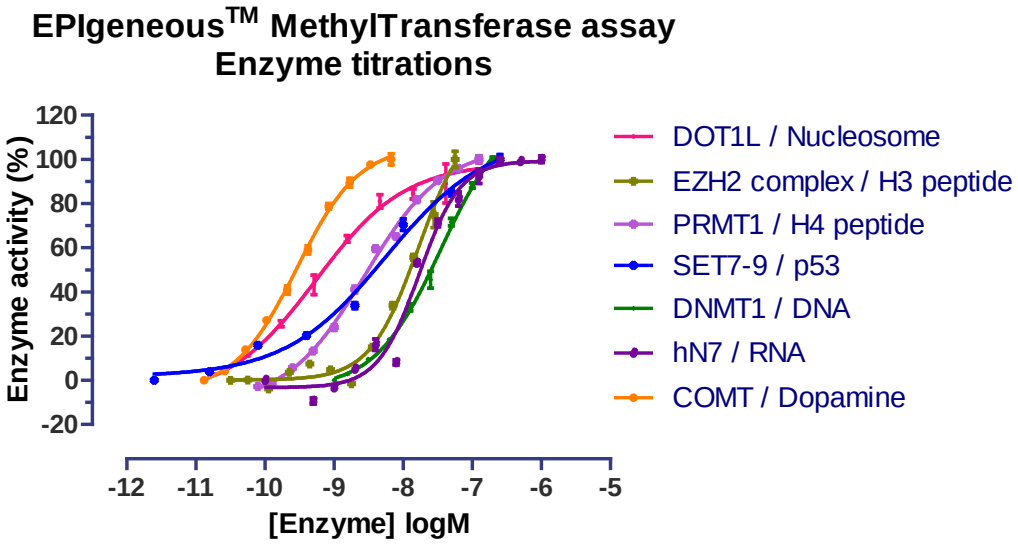
short-life fluorescence (when not engaged in FRET) (ns)

Temporal selectivity



EPIgeneous™ Methyltransferase assay

A large set of validated enzymes and substrates



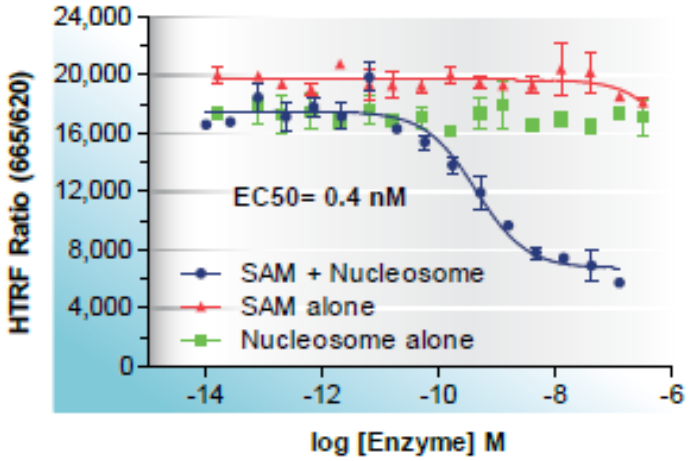
Methyltransferases	Substrates
G9a, EZH2 complex, SET7/9	H3 (1-21) or (1-50) peptide
PRMT1	H4 (1-25) peptide
DOT1L, SETD2, MLL1 complex	Oligonucleosome
SET7/9	p53
DNMT1	DNA (poly(dI-dC))
hN7, WNV, NSP14, NSP10-16	RNA
COMT	Dopamine

- Assay validated with 13 different enzymes on 7 different substrate types:
  - H3 peptide
  - H4 peptide
  - Oligonucleosome
  - P53
  - DNA
  - RNA
  - Dopamine

EPIgeneous™ Methyltransferase Assay

DOT1L optimization

1. DOT1L titration



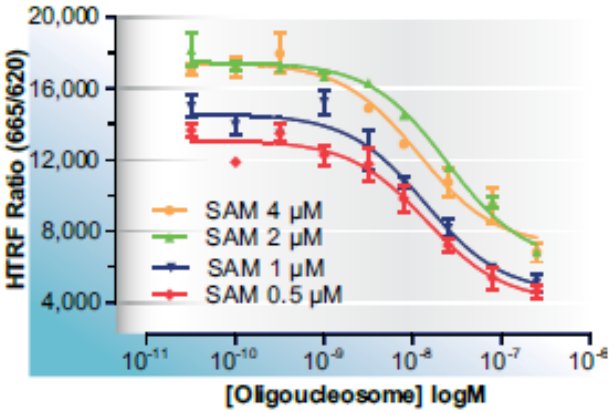
Detection of DOT1L specific activity and identification of optimal enzyme concentration. Human recombinant DOT1L was serially diluted to the indicated concentrations and the assay carried out with 10ng/µl (= 77 nM) oligonucleosome as substrate and 2 µM SAM for 2 h at 30°C. The negative controls (no SAM or no nucleosome) show the measurement of the enzymatic specific activity.

A DOT1L concentration of 4.5 nM (EC80) was selected for further experiments. This concentration lead to 10% conversion of SAM into SAH

EPIgeneous™ Methyltransferase Assay

DOT1L optimization

2. Oligonucleosome and SAM titrations



Determination of optimal substrate and SAM concentrations.

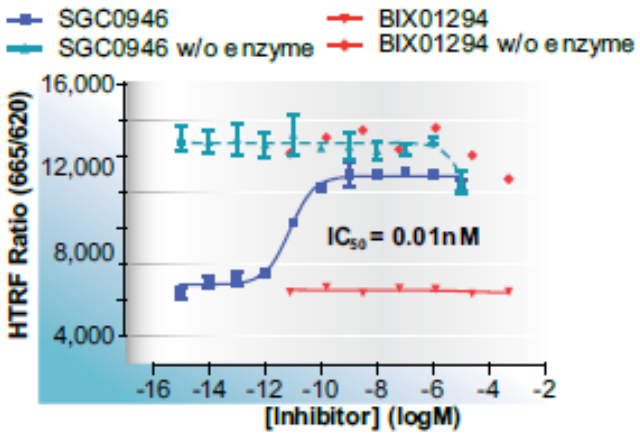
Oligonucleosome was titrated with several concentrations of SAM. DOT1L is used at 4.5 nM and incubated with SAM and substrate 2h at 30°C.

0.5µM of SAM, a concentration below reported Km of 0.67µM (1), is selected for subsequent experiments. For oligonucleosome, 77 nM (EC80) is selected for further tests.

EPIgeneous™ Methyltransferase Assay

DOT1L optimization

3. Inhibitor titration



EPIgeneous™ methyltransferase assay validated by measuring activity of SGC0946 inhibitor.

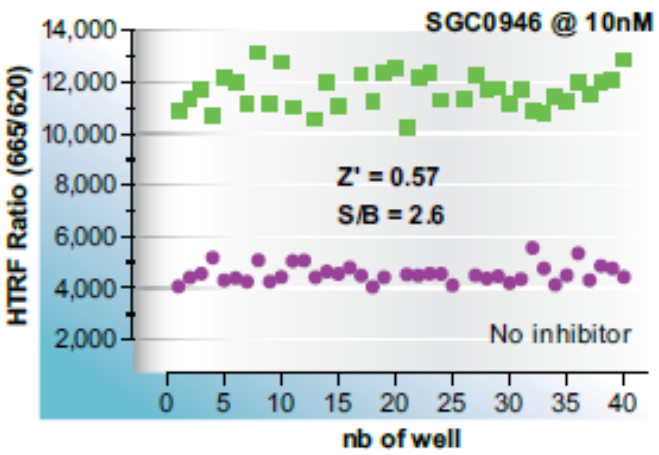
This assay was performed using 0.5 µM SAM (EC40), 77nM oligonucleosome (EC80) and 4.5 nM DOT1L (EC80). The enzymatic reaction was stopped with the detection reagents after a 2h incubation at 30°C.

- IC50 of SGC0946 is in good agreement with the literature (2).
- As expected, BIX01294 which is a G9a selective inhibitor does not inhibit DOT1L.
- Controls of inhibitors without enzyme show that they do not affect the detection reagents.

EPIgeneous™ Methyltransferase Assay

DOT1L optimization

4. Z' factor



Assay robustness demonstrated through Z' factor determination.

This assay was performed using 0.5 µM SAM (EC40), 77nM oligonucleosome (EC80) and 4.5 nM DOT1L (EC80).

The Z' factor was obtained with biological balanced conditions and underlines the robustness of the assay and its suitability for HTS in biological relevant conditions.

EPIgeneous™ Methyltransferase Assay

An adaptable assay for substrates, enzymes, inhibitors and SAM concentrations

- **Validated for 13 methyltransferases**
- **Validated on 7 substrates (peptides, core histones and nucleosomes, DNA, RNA, small molecules and proteins such as p53)**
- **Compatible with a wide range of SAM concentrations (0.4 – 200 µM)**
- **Work in balanced biological conditions with a robust assay (Z' = 0.57 – 0.78 @ EC_{80} enzyme, SAM & substrate)**
- **Direct detection eliminates the need for coupling steps and reduces false positives**
- **No need for site specific or modification specific antibodies reducing additional assay development cost and time**


Adapt to anything with a truly universal assay
earn more about it
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ASSAYS
INTERACTION IS EVERYTHING.

Thank you