**EPigeneous™ Methyltransferase assay: a new HTRF Universal SAH detection assay to assess methyltransferase activity**


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**INTRODUCTION**

Epigenetics is one of the fastest growing fields in research because of their potential to identify new drugs for therapeutic targets and especially in oncology. A key focus of epigenetics research is on methyltransferases because of their abundance and ability to methylate histones and various other substrates. Cisbio has developed a universal methyltransferase mix and read assay using HTRF technology that provides the sensitivity and flexibility of substrates. This assay, which directly quantifies S-5'-adenosyl-L-methionine (SAM), has been successfully validated on a variety of enzymes and substrates (see table). This study presents the principle, protocol and specifications of the assay as well as assay performances and optimization on an enzyme of interest: DOT1L with nucleosome as the substrate.

**DOT1L / Oligonucleosome assay**

1. **DOT1l titration**

   The assay consists of an enzymatic and detection step. The methyltransferase activity is assessed by measuring the conversion of SAM (S-5'-adenosyl-L-methionine) to SAH. In order to directly measure SAM release, an anti-SAM antibody labeled with terbium cryptate and a SAH-d2 tracer were used. The SAH released by the enzymatic reaction competes with the SAH-d2 labeled leading to a decrease of the HTRF signal. Oligonucleosome and all methyltransferase enzymes, but those for RNA and dopamine substrates, were provided by Reaction Biology Corp.

2. **Oligonucleosome and SAM titrations**

   Oligonucleosome was titrated with several concentrations of SAM. DOT1L is used at 4.5 nM and incubated with SAM and substrate 2 h at 30°C. 0.5 µM of SAM, a concentration below reported Km of 0.67 µM (1), is selected for subsequent experiments. For oligonucleosome, 77 nM (EC80) is selected for further tests.

3. **Inhibitor titration**

   A DOT1L concentration of 4.5 nM (EC80) is selected for further tests.

**Validated Methyltransferases**

- **NLII complex / Nucleosome**
- **DOT1L / Nucleosome**
- **SETD2 / Nucleosome**
- **G9a / HO peptide**
- **G9a / 21 peptide**
- **EZH2 complex / HO peptide**
- **SET7-9 / HO peptide**
- **SET7-9 / p53**
- **COMT / DNA**
- **COMT / Oligonucleosome**
- **MLL1 complex / Nucleosome**
- **PRMT1 / H4 peptide**
- **PRMT1 / HO peptide**
- **PRMT1 / DNA**
- **PRMT1 / Oligonucleosome**
- **NSP10-16 / RNA**
- **NSP10-16 / DNA**
- **NSP10-16 / Oligonucleosome**
- **hN7 / RNA**
- **WNV, NSP14, NSP10-16 RNA**
- **G9a, EZH2 complex, SET7/9 H3 (1-21) or (1-50) peptide**
- **MLL1 complex / Nucleosome**
- **PRMT1 / H4 peptide**
- **PRMT1 / HO peptide**
- **PRMT1 / DNA**
- **PRMT1 / Oligonucleosome**
- **G9a Recombinant full H3**
- **SET7-9 / p53**
- **COMT / Dopamine**

**CONCLUSION**

We have developed a universal methyltransferase mix and read assay using HTRF technology that provides:

- The flexibility of substrates. The assay is validated with a large set of methyltransferase sub families so far: PKMT and PRMT on histone peptides, nucleosomes or other proteins (p53) / DNA MT; RNA MT and COMT with dopamine.
- Non radioactive assay with high sensitivity by measuring the release of SAM. Avoid false positives and counter screening due to coupling enzymes and indirect measurement format.
- Flexible enzymatic assay conditions: large SAM concentration range compatibility (0.4 – 200 µM).

**REFERENCES**

(2) Yu et al. Nature commun., 2012