Detection of JumonjiC Domain-Containing Histone Demethylase activities With a Homogeneous Bioluminescent Succinate Assay

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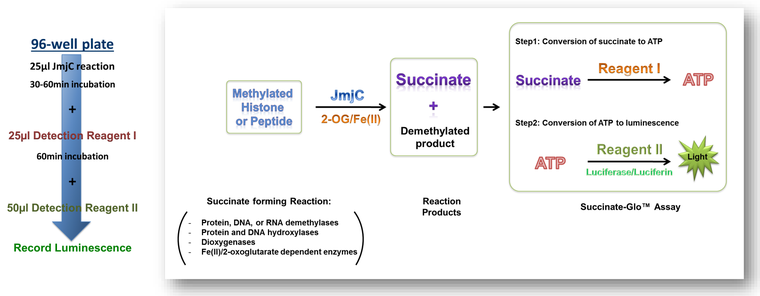
1. Introduction

JumonjiC domain-containing histone lysine demethylases (JMJCs) play a pivotal role in determining the epigenetic status of the genome, leading to either transcriptional repression or activation of target genes by counteracting the activities of histone lysine methyltransferases. Because of their implication in cancer, they have become validated drug targets. Therefore, assays for this enzyme subfamily are desirable in order to facilitate the identification of selective and potent inhibitors for drug discovery and as basic research tools. Since succinate is one of the products of the demethylation reaction catalyzed by these enzymes, an assay that detects succinate would be suitable for monitoring all JMJC activities as well as other succinate-forming enzymes (i.e.: dioxygenases). Thus, a bioluminescent and homogenous succinate detection assay for measuring JMJC activity was developed, and it is performed in a two-step format that relies on converting the succinate product to ATP, and then to light in a robust luciferase reaction. The light output is proportional to succinate concentration from low nM to 15µM, and the assay is highly sensitive and robust, two features that are highly desirable and essential for measuring the activity of the majority of JMJC demethylase subfamilies. Therefore, the succinate detection assay is a simple-to-use method that does not require antibodies or modified substrates. Examples of various applications of this succinate detection assay will be presented, including studies on specificity of different substrates by diverse JMJCs, as well as mode of action studies using specific inhibitors. The development of this succinate detection assay will make it possible to investigate a large number of JMJC demethylases and could have significant impact on diverse areas of Epigenetics research.

2. Succinate Detection Assay Principle

Principle: Succinate is converted into ATP that is detected via a luciferase/luciferin reaction

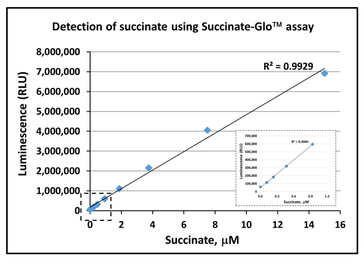


White Plates	Format 1:1:2 (µl)	
384 wells	5/5/10	10/10/20
96 wells	25/25/50	50/50/100

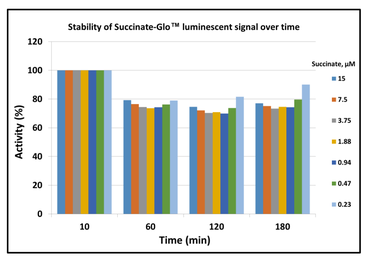
- Two Step Detection: After the demethylase reaction, the Succinate Detection Reagents are added in 1:1:2 ratio
- Luminescence signal is proportional to the succinate produced and to the demethylase activity
- Simple “Add and Read”: No radioisotopes. No product separation. No antibodies

3. High Sensitivity, Linearity and Signal Stability

Linearity



Signal Stability



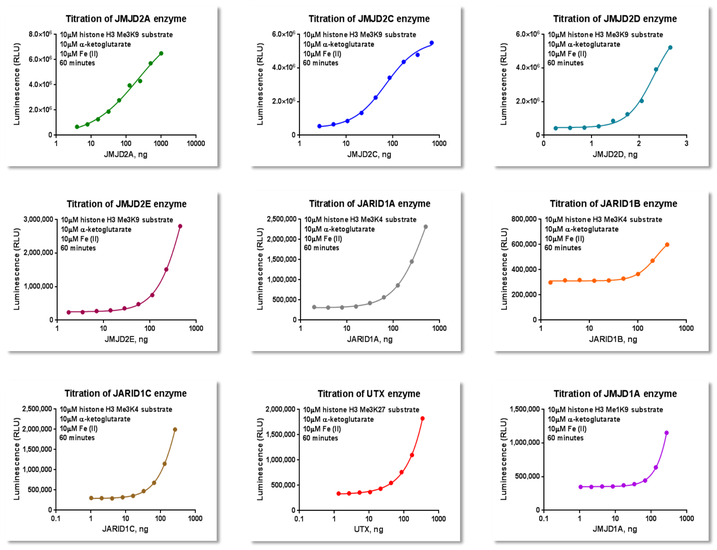
Sensitivity

Signal to Background ratios at different succinate concentrations									
Succinate, µM	15	7.5	3.75	1.88	0.9375	0.47	0.234	0.12	0
S/B values	118	69	37	19	10	5	3	2	1

- Succinate detection is linear up to 15µM and it has a high dynamic range
- It can detect as low as 200nM with a signal/background (SB) of 2
- The signal is stable for up to 3 hours with ~80% remaining signal

4. Universal Activity Assay for Several JMJC Demethylases

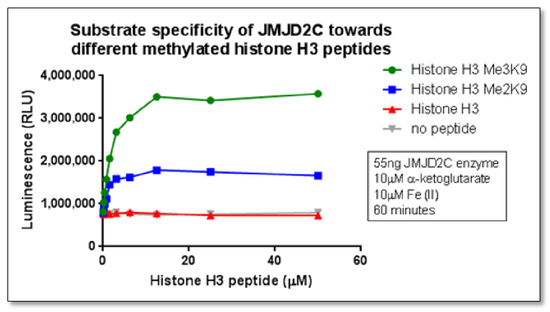
Succinate-Glo™ is one assay for diverse JmjC demethylase-substrate combinations.

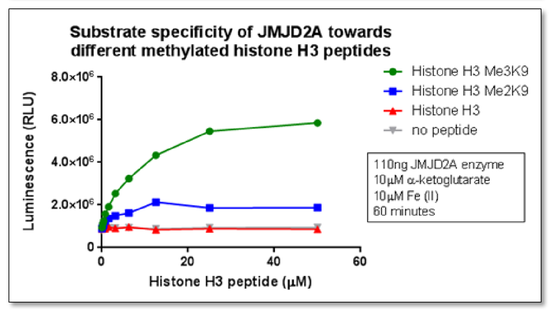


Succinate detection is suitable for measuring the activity of any JMJC demethylase regardless of substrate methylation state and position

5. JMJC Demethylase Substrate Specificity Studies

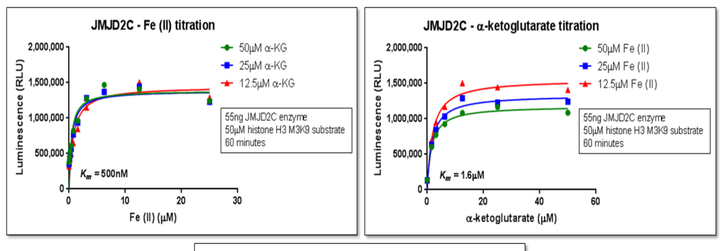
Succinate detection can be used to study JMJC demethylase specificity towards diverse methylated substrates





6. Evaluating Kinetic Parameters Using Succinate Detection

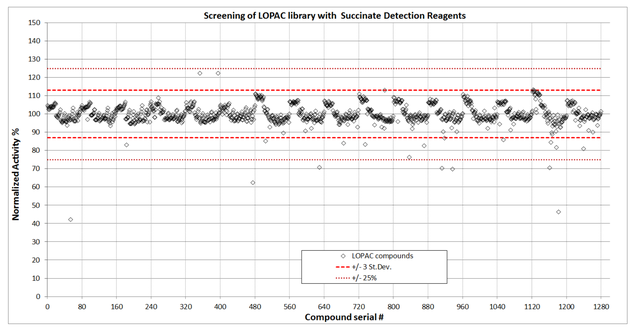
Succinate detection can be used to evaluate the kinetic parameters of different JMJC demethylase substrates



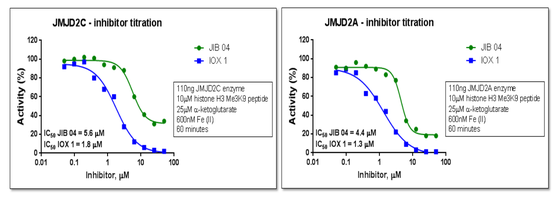
- Km values for substrates and cofactors detected with Succinate-Glo™ assay are similar to the ones reported in literature.

7. JMJC Demethylase Inhibitor Studies

LOPAC screening



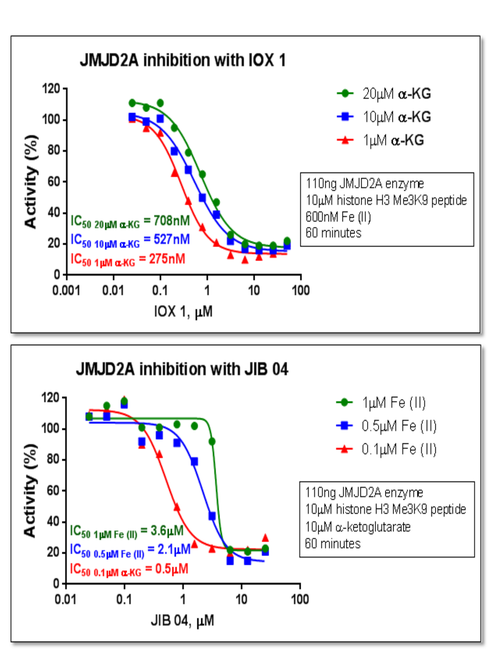
Demethylase inhibitors



- Small molecule inhibitors do not interfere with succinate assay detection
- JMJC activity detected with succinate assay is inhibited with selective inhibitors

8. Mode of Action Studies

Bioluminescent succinate assay can be used to evaluate competitive inhibitors toward different substrates



9. Conclusions

Universality:

- Bioluminescent succinate assay can be used with the majority of JMJC demethylases
- One assay for diverse JMJC demethylase-substrate combinations, regardless of substrate methylation state and position

Versatility:

- Easy to use assay. 2-step addition and read
- Suitable for studying substrate specificity, kinetic parameters and mode of action of JMJC demethylase inhibitors

HTS friendly:

- Sensitive in low volume format
- Signal is stable for batch processing
- Resistant to chemical interference

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