The covalent modifications of histone proteins, DNA and RNA by Fe(II)/2-oxoglutarate-dependent dioxygenases are key to the modulation of biological processes such as epigenetics, hypoxic signaling and DNA/RNA repair. Of these, Jumonji domain-containing histone lysine demethylases (JMJs), the ten-eleven translocation (TET) DNA dioxygenases, the ALKB DNA/RNA hydroxylases and the prolyl hydroxyrases EGLN1-3 have generated increased interest as potential drug targets for the treatment of a number of pathological conditions, including cancer. Therefore, there is a strong need for biochemical assays to monitor the activity of this class of enzymes, study their modes of regulation, and to search for selective and potent inhibitors without relying on cumbersome technologies such as radiometry or antibody-based methods. Since succinate is a common product to all Fe(II)/2-oxoglutarate-dependent dioxygenases, we determined the substrate specificities, the apparent kinetic constants and the inhibitor’s mode of action for members of the dioxygenase superfamily, as well as inhibition profiles of reported inhibitors using a novel bioluminescent and homogenous succinate detection assay. Our results demonstrate that a universal succinate detection is a useful strategy for the characterization of multiple Fe(II)/2-oxoglutarate-dependent dioxygenases with distinct substrate requirements, enabling the investigation of a large number of enzymes and their modulators that cannot be evaluated in a miniaturized or high-throughput manner with currently available methods.

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

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

Succinate detection can be used to evaluate the kinetic parameters of different 2-oxoglutarate dioxygenases.

Succinate detection can be used to measure the activity of JMJC demethylase regardless of substrate methylation state and position.

Succinate detection can be used to measure the activity of 2-oxoglutarate dependent dioxygenases and hydroxylases.

Succinate detection proved here to be a useful strategy for the characterization of diverse Fe(II)/2-oxoglutarate-dependent dioxygenases. The Succinate-Glo assay has the following advantages over currently available technologies:

Universality:
- This assay can be used with the majority of JMJC demethylases and 2-oxoglutarate dioxygenases regardless of substrate chemical structure, methylation state or position

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

HTS friendly:
- Sensitive in low volume format and signal is stable for batch processing
- Resistant to chemical interference and suitable for inhibitor studies

Bioluminescent succinate detection proved here to be a useful strategy for the characterization of diverse Fe(II)/2-oxoglutarate-dependent dioxygenases. The Succinate-Glo assay has the following advantages over currently available technologies:

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