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BV 2011-07 - *New Assay Kit to Quantitatively Measure Methyltransferases and Demethylase Activity in Gene Regulation*

SUMMARY:

The proposed invention will provide a competitive advantage to any company or organization involved with the regulation of gene expression, cancer research, rational drug design, or any research and development relating to methyltransferase and demethylase activities. The impact of histone and 11011-histone modification on gene expression and organism developmental programs is currently an active area of research. The invention is a quantitative assay that can differentiate between polypeptides having no methyl, monomethyl, dimethyl, and optionally trimethyl groups on defined lysine residues. The assay can simultaneously gather quantitative measurements of methylation in any of the four possible states (un-, mono-, di-and tri-). Combinations of these modifications impact the regulation and developmental programs in gene expression. Histone modifications through methylation have been shown to be very important in gene expression and cancer research. The enzymes capable of adding a methyl group (methyltransferase) or removing a methyl group (demethylase) are of central interest because they arc differently regulated in a plethora of cancers. The demethylase LSD I is associated with gene repression and has been suggested to be important in initiating myc-induced transcription in cancers. Structural and biochemical studies have led to the development of numerous LSD! inhibitors that have the potential to be important therapeutic tools. In addition, the mechanism of LSD I indicates that it is an excellent candidate for suicide inhibitors. Many monoamine oxidase inhibitors (MAO!s) have been suggested as potential LSD I suicide inhibitors. Obtaining a quantitative measurement of the demethylase LSD I is of critical importance for research, however with current technology the demethylase activity can only be measured semi-quantitatively. Methylation can occur in four different states (un-, mono-, di-and tri-) in a single sample. Deciphering, quantitation, and comparing these different methylated states is essential for a full understanding of the regulatory signals required. An assay with the ability to measure each of these different methyl states quantitatively within a single population is currently not available. The present assay overcomes these. Currently histone demethylase activity can be measured in several different ways; with fluorography an enzyme can be monitored to determine if it is a demethylase, but it is not quantitative in nature. Another common method is with western blotting using antibodies specific to certain modifications, but the antibodies used

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for histone modifications are non-specific making absolute quantification difficult. An alternative is mass spectrometry, but the analysis is at best semi-quantitative because each of the different states of methylation will not ionize predictably for analysis. The present analytical methods overcome this in distinguishing the four different states of methylation. This assay would prove invaluable to fu1iher the study of not just LSD I but also many other demethylase enzymes and the efficiency of their proposed inhibitors, and would have an immediate impact in cancer research.