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Using Multiplexed ELISA to Obtain an Acetylation Profile of Three Core Histones and p53

Authors: Chris Lyman[1], Brett Baumgartner[1], Terrance Lam[2]

[1]Quansys Biosciences, Logan, UT, [2]Cell Signaling Technology, Danvers, MA

Abstract:

Modifications such as acetylation, phosphorylation and methylation of histone proteins have an effect on chromatin structure. Altering chromatin structure changes the accessibility of chromatin to transcription factors involved in gene expression.

The objective of this study was to characterize the acetylation profile on histone proteins and p53 using multiplex ELISA. Researchers previously relied on Western Blot and ELISA assays to determine the presence of acetylated protein. This study used a multiplex ELISA platform to determine whether histone proteins (H2A, H3, H4) and p53, were being acetylated in response to treatment with stimuli. Two cell lines were stimulated with Trichostain A (TSA) overnight and the resulting lysates were run in a multiplex ELISA assay.

The results show that COS7 and 3T3 cells treated with TSA both exhibit positive responses for H2A, H3, H4 and p53. Untreated 3T3 cells show a significant reduction of acetylated histones and acetylated p53. Untreated COS7 cells did not produce acetylated histone proteins but did produce acetylated p53.

TSA treatment of 3T3 and COS7 cell lines induces acetylation of histone proteins and p53. Using multiplex ELISA allowed the researcher to obtain acetylation information for four proteins simultaneously.

Introduction:

Gene expression is one of the most tightly controlled processes that take place within a cell. Promoters, transcription factors, and RNA processing are all key factors that facilitate gene expression control. In addition to these factors, modifications to the histones in which the DNA is wrapped around is critical in maintaining proper gene expression. Histones can undergo modification; including methylation, acetylation, and phosphorylation. Acetylation of the lysine residues of histone proteins reduces the affinity between histones and DNA. This altering of the chromatin structure increases the accessibility of RNA polymerase and transcription factors to the DNA. Therefore, histone acetylation enhances transcription. The deacetylation of histones re-establishes the affinity of the histone for DNA reversing the accessibility of RNA polymerase and transcription factors.

In addition to histone acetylation many transcription factors may also undergo acetylation. One key transcription factor that undergoes acetylation is p53. As a tumor suppressor p53 plays a crucial role in cellular response to DNA damage. Activated p53 can lead to both cell cycle arrest and DNA repair or to apoptosis. There are multiple modes of action on p53. Many include the phosphorylation at various sites on the p53 molecule. Additionally when p53 is acetylated on at Lys382 its ability to bind DNA is enhanced.

The goal of this study was to characterize the acetylation profile on histone proteins and p53 using multiplex ELISA. Traditionally these profiles are determined by either a western or an ELISA. Using Quansys' Multiplex ELISA technology this study was able to simultaneously in one test determine whether or not histones H2A, H3, H4, and p53 were acetylated in COS7 and 3T3 cell lines when stimulated with Trichostain A (TSA).

Materials and Methods:

NIH/3T3 and COS7 cells were grown in ATCC recommended conditions and media. Once the cells were near confluent they were then treated with 400nM trichostain A (TSA) overnight. Post treatment the cells were harvested and lysed. The cell lysates were then run on Quansys Multiplexed ELISA assay for Histone and p53 acetylation.

The Multiplexed ELISA consist of four unique capture antibodies each specific for p53, H2A, H3,and H4 spotted in an array format in each well of a 96-well plate. The sample was incubated for one hour in the plate as with a traditional ELISA. Following sample incubation a detection reagent is added that contains a specific antibody for detection of acetylation on p53, H2A, H3 and H4. This step is then followed by incubation with a chemiluminesent conjugate and imaging with a CCD camera. The resulting light produced at each capture spot location correlates with the amount of acetylated histone or p53 present in the sample.

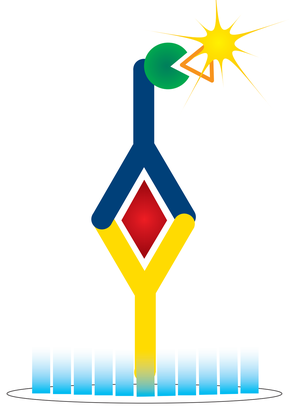

Figure 1: Sandwich ELISA

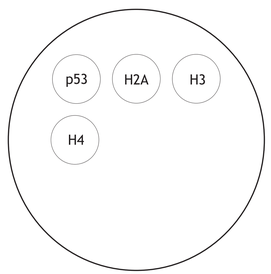

Figure 2: Spot location in each well

Results:

Both treated and untreated COS-7 cell lysates contained acetylated p53. Although it would appear that the untreated COS-7 cells had more acetylated p53 than did the treated cells. Conversely it would appear that TSA treatment enhanced p53 acetylation in the NIH/3T3 cells. The three histones included in the assay (H2A, H3, and H4) showed marked increase in acetylation in both the Cos-7 and NIH/3T3 cell when treated with TSA.

Conclusion:

TSA increases histone H2A, H3, and H4 acetylation in NIH/3T3 and Cos7 cell lines. P53 acetylation was also increased in 3T3 cells but decreased in COS 7 cells. Quansys Multiplexed ELISA technology allowed for more rapid and accurate determination of acetylation profiles relative to traditional methods which would have required multiple hours if not days to acquire comparable data. In the quest to more fully understand the cell cycle and cell signaling Multiplex ELISA technology is an invaluable and progressive research tool.

Relative Acetylation Levels

	NIH/3T3 Lysate		COS7	
	TSA Treated	Untreated	TSA Treated	Untreated
p53	63951	2017	13561	63941
H2A	49420	2215	30315	6021
H3	63954	5089	63954	16434
H4	63952	4154	39641	10362

Chart 1: Pixel Intensity

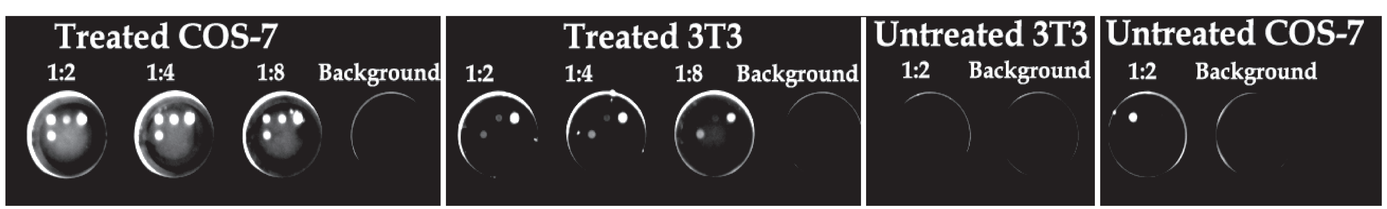

Figure 3: Multiplex ELISA image