Bio-Plex™ suspension array system tech note 5377

Lysate Preparation for Bio-Plex Phospho-Histone H3 Assay

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Introduction

The Bio-Plex phospho-histone H3 assay provides an excellent indicator for cell cycle progression and activation or silencing of distinctive signal transduction pathways in lysates derived from cell culture or tissue samples. Phosphorylation at Ser^{10} of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis in the cell cycle. Because of the unique physical properties of histone H3 molecules, traditional lysate preparation as stated in the Bio-Plex cell lysis product kit insert did not yield significant signal for the phospho-histone H3 assay. Therefore, a modification to the existing protocol was explored using the M phase of HeLa cells.

Subjecting the HeLa M phase lysate to sonication and a freeze/thaw cycle showed a significant increase in the performance of the phospho-histone H3 assay in terms of median fluorescence intensity (MFI), sensitivity, and correlation with protein concentration. We were also able to address the following questions related to Bio-Plex phospho-histone H3 assay:

- Will the modification to the existing lysate preparation protocol apply to other cell lines for the detection of phospho-histone H3?
- If it does, will the phospho-histone H3 assay with the M phase lysates of other cell lines be commensurate with the performance of the HeLa/M lysate?

In this tech note, we describe the protocol for cell lysate preparation and present the data from the Bio-Plex phospho-histone H3 assay with the cell lysates from both HeLa and COS-7 cells.

Methods

Cell Line Determination

The HeLa cell line, which originated from epithelial cells isolated from a human cervical adenocarcinoma, is a well-characterized cell line in cell cycle research. Using HeLa cells as a model cell line, we developed a procedure for preparing an M phase lysate for phospho-histone H3 (Figure 1). The cells were coerced into a synchronized M phase cell cycle. The COS-7 cell line was also used to verify the procedure.

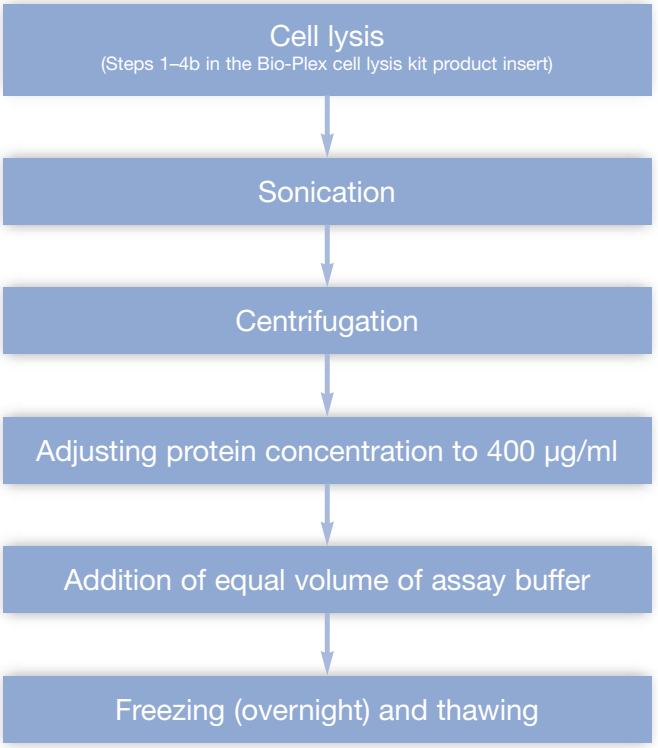

Fig. 1. Overview of the modified lysate preparation procedure.

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Protocol for Cell Lysate Preparation

We prepared four treatments of HeLa and COS-7 M phase cell lysates:

1. Traditional preparations.
The lysates of M phase cells were prepared according to the instructions specified in the Bio-Plex cell lysis kit product insert.

2. Sonicated.
The lysates of M phase cells were prepared according to the instructions specified in the Bio-Plex cell lysis kit product insert. Additionally, the lysates were subjected to sonication before centrifugation (step 4b of the Bio-Plex cell lysis kit product insert). The sonication condition was 10 pulses, 1 min break, and 10 pulses again with a Sonifier S-450 (Branson Ultrasonics Corporation) set at duty cycle 40 and output level 1.

3. Frozen/thawed.
The lysates of M phase cells were prepared according to the instructions specified in the Bio-Plex cell lysis kit product insert. Additionally, the lysates were subjected to a freeze (overnight)/thaw cycle after being diluted to a final concentration of 200 µg/ml with an equal volume of assay buffer.

4. Sonicated and frozen/thawed.
The lysates of M phase cells were prepared by combining the sonication and freeze/thaw treatments.

Bio-Plex Assays and Western Blotting

The lysates were evaluated for phospho-histone H3 detection according to the Bio-Plex phosphoprotein assay instructions. Western blot analysis was conducted using the same amount of lysate for correlation studies.

Results and Discussion

Sonication Improved Sensitivity

Two sets of HeLa/M and COS-7/M lysates were prepared. Both sets were subjected to a freeze/thaw cycle but only one was sonicated. The phospho-histone H3 assay showed that the sonicated HeLa/M and COS-7/M lysates yielded 3- to 4-fold higher in MFI value than the frozen-thawed lysates without sonication (Figure 2), suggesting more sensitive detection of phospho-histone H3 with the sonicated M phase cell lysates.

Freeze/Thaw Treatment Enhanced Detection

Two sets of HeLa/M and COS-7/M lysates were prepared; both sets were sonicated but only one was subjected to a freeze/thaw cycle. The phospho-histone H3 assay showed that the frozen/thawed HeLa/M and COS-7/M lysates yielded 5- to 7-fold higher MFI values than those without a freeze/thaw cycle (Figure 2), suggesting that the freeze/thaw cycle significantly improved the signal of the M phase lysates.

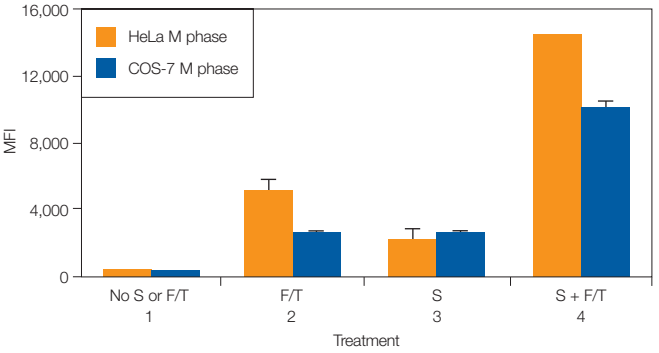

Fig. 2. Sonication and freeze/thaw treatment. The Bio-Plex phospho-histone H3 assay showed improvement with the use of sonication and a freeze/thaw treatment of the lysates. (S = sonication; F/T = freeze/thaw.)

Correlation Between the Bio-Plex Assay and Western Blot Analysis

HeLa/M and COS-7/M lysates were prepared by sonication and a freeze/thaw cycle, and the lysates were diluted to protein concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.313 µg/sample. Samples were tested with Bio-Plex phospho-histone H3 assays. The same amount of protein was used in western blot analysis. The results are presented in Figure 3. For both HeLa and COS-7 M phase cell lysates, the MFI value from the Bio-Plex assay correlated with the intensity of protein bands in western blot analysis. Similar dose-responses were detected in both the Bio-Plex assay and western blot analysis with both lysates. These results suggest a high quality of M phase lysates prepared in the modified procedure for the phospho-histone H3 assays in terms of sensitivity and dose-response.

Conclusions

Though preparing lysates as specified in the Bio-Plex cell lysis product kit insert generated adequate MFI signals and sensitivity for most Bio-Plex phosphoprotein and total target assays, it did not have comparative performance for the Bio-Plex phospho-histone H3 assay. In this study, we found that sonication and a freeze/thaw treatment can increase the extent of cell lysis, leading to efficient detection of phospho-histone H3 in the Bio-Plex assay.

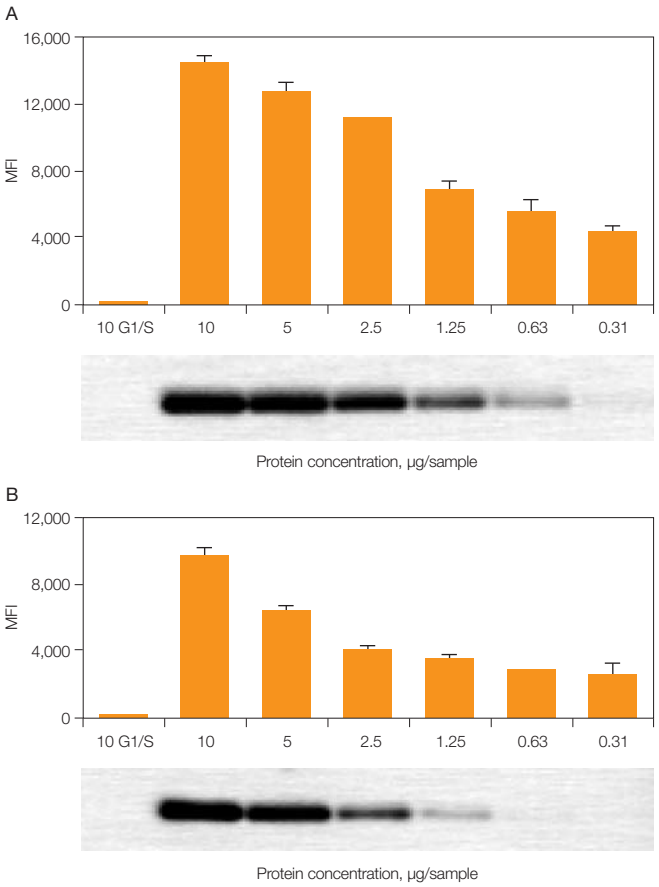

Fig. 3. Correlation of Bio-Plex phospho-histone assays and cell lysate dose-response with western blots. A, HeLa M phase lysate; **B**, COS-7 M phase lysate. G1/S, G1/S phase control.

- This elevated performance in the Bio-Plex phospho-histone H3 assay requires the combination of sonication and a freeze/thaw cycle; additionally, the full freeze/thaw effect requires the presence of an equal volume of assay buffer in the lysates during the freeze/thaw process
- The modified procedure applies to the preparation of other cell lysates tested with the phospho-histone H3 assay
- Lysates prepared using this modified protocol demonstrated good sensitivity and correlation with western blot analysis

The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

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