Bio-Plex® suspension array system

tech note 3141

Detection of Phosphorylated and Total ERK and p38 MAPK by Bio-Plex Assay, ELISA, and Western Blotting

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Introduction

Western blotting is a traditional technique for detecting phosphorylated proteins within a variety of cell culture and tissue sample lysates. In recent years, new techniques that apply a sandwich antibody capture approach have become available for detecting phosphorylated proteins. These techniques include Bio-Plex phosphoprotein assays, which are multiplex bead-based assays for the detection of one or more different phosphorylated proteins within a single sample. Enzyme-linked immunosorbent assays (ELISA) are another technique that has been increasingly applied to detect phosphorylated proteins a single target at a time. In this study, commercially available Bio-Plex phosphoprotein and total target assays, and phosphoprotein and total ELISA assays, are compared with western blotting in terms of sensitivity, detection range, and level of correlation, for the detection of phospho- and total ERK and p38 MAPK.

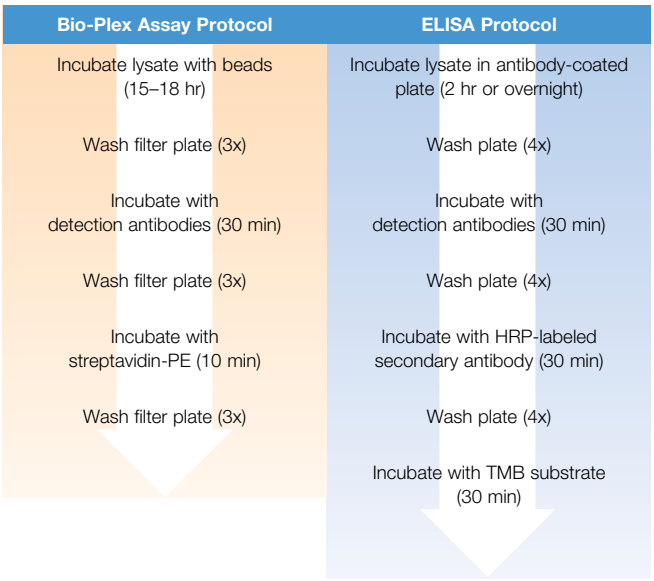
Methods

Lysate Preparation

Lysates of HEK 293 and HeLa cells were prepared according to two techniques. The Bio-Plex cell lysis kit was used to prepare cell lysates for western blot and Bio-Plex analysis (refer to bulletin 3033). A different lysing protocol specified in the ELISA kit vendor's manual was followed to prepare cell lysates for ELISA.

Assay Procedure

The procedure detailed in bulletin 3033 was followed for western blot analysis. The procedure provided in the Bio-Plex phosphoprotein detection reagent kit was followed for the Bio-Plex phosphoprotein and total target assays (summarized in the flowchart opposite). The procedure provided in the ELISA kit vendor's manual was followed for the phospho- and total protein ELISAs. The same antibodies specific to each target were used in both western blot and Bio-Plex phosphoprotein and total target assays. The antibodies used in the ELISAs were those provided in the vendor kit.



Results and Discussion

Total p38 MAPK

Total p38 MAPK was detected by ELISA in HEK 293 lysates with protein concentration ranging from 20 µg/well to 0.625 µg/well and absorbance ranging from 2.623 to 0.317 (Figure 1A). The limit of detection appears to be around 0.625 µg/well. A similar dose response was observed with the Bio-Plex assay; however, an MFI value of 897 at the lowest data point (0.625 µg/well) suggests the potential to detect further-diluted samples (Figure 1B). The data also suggest high-end saturation over 10 µg/well. With the western blot, the target was detected down to a protein concentration of 1.25 µg/lane (Figure 1C).

Total p38 MAPK was not detected, nor was a dose response observed with ELISA of HeLa lysates, even at the highest protein concentration tested (Figure 1D). This target was, however, detected with the Bio-Plex assay in each sample, with the MFI ranging from 4,950 to 321 (Figure 1E), which correlated well with the western blot (Figure 1F).

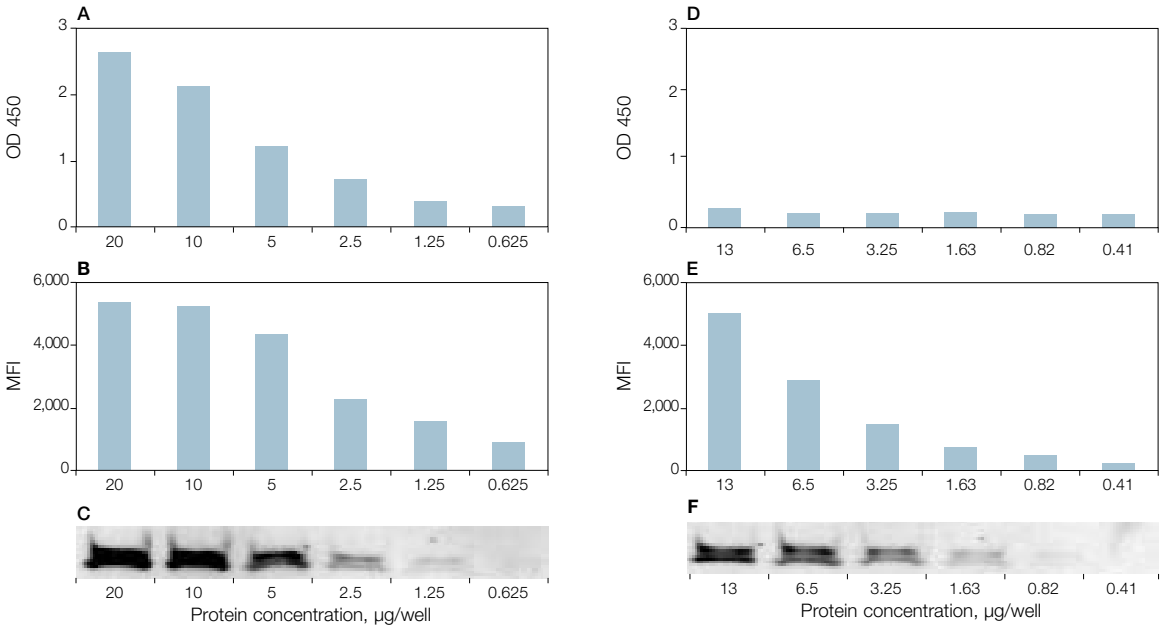


Fig. 1. Total p38 MAPK detection. A–C, HEK 293 cell lysate; **D–F**, HeLa cell lysate. **A** and **D**, ELISAs; **B** and **E**, Bio-Plex assays; **C** and **F**, western blots.

Phospho-p38 MAPK

Phospho-p38 MAPK (Thr^{180}/Tyr^{182}) was detected by ELISA in UV-treated HEK 293 lysate with protein concentration ranging from 12 µg/well to 0.75 µg/well (Figure 2A). The ELISA displayed a high-end saturation plateau in samples with a protein concentration from 12 µg/well to 3 µg/well (Figure 2A), which suggests that further dilution is necessary to detect a dose response. The Bio-Plex assay showed a distinct dose response (Figure 2B) that correlated well with the western blot (Figure 2C).

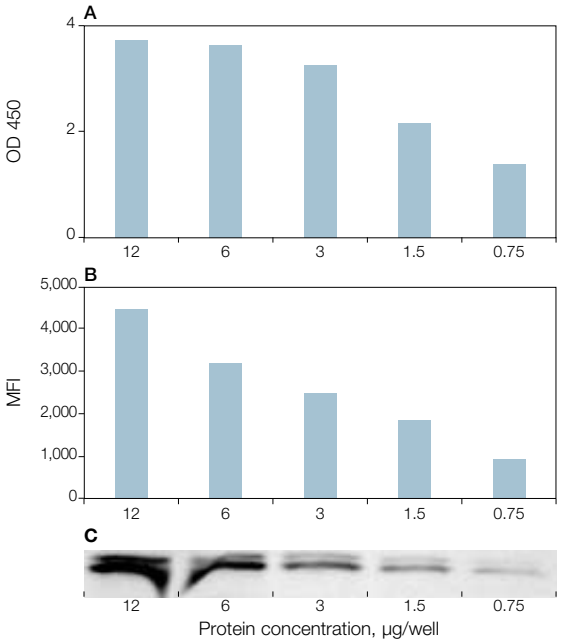


Fig. 2. Phospho-p38 MAPK detection. Samples were UV-treated HEK 293 cell lysates. **A**, ELISA; **B**, Bio-Plex assay; **C**, western blot.

Total ERK

Total ERK1/2 was detected by ELISA in HEK 293 lysates with protein concentration ranging from 10 µg/well to 0.625 µg/well and absorbance ranging from 0.331 to 0.160 (Figure 3A). To explore the Bio-Plex total ERK2 assay sensitivity further, lysates were further diluted to a protein concentration range of 1.25–0.078 µg/well (Figure 3B). In comparison, a faint band was visible on the western blot at a total protein concentration of 0.625 µg/lane (Figure 3C).

Both sandwich antibody methods detected total ERK in HeLa lysates with protein concentration ranging from 13 µg/well to 0.41 µg/well. A flattened lower-end dose response was observed with the ELISA, and an absorbance range of 0.500–0.158 showed a limited ability to distinguish between different samples (Figure 3D). The Bio-Plex total ERK2 assay better distinguished signal intensities from the high end to the low end, with MFI from 11,713 to 603 (Figure 3E). The western blot band became faint at 3.25 µg/well, indicating the level of sensitivity compared to the Bio-Plex assay (Figure 3F).

Phospho-ERK

Epidermal growth factor (EGF)-treated HEK 293 cell lysates were used for phospho-ERK (Thr^{202}/Tyr^{204}) assay comparison. Lysates were prepared using the Bio-Plex cell lysis kit or by following a protocol provided with the ELISA kit. ELISA showed a limited ability to distinguish the first two samples, with total protein concentrations of 2 µg/well and 1 µg/well; the reported sample absorbance reached a plateau, and the highest OD reported was only 1.755 (Figure 4A). The Bio-Plex assay demonstrated distinctly separate signals across all the samples (Figure 4B), which correlated well with the western blot bands (Figure 4C).

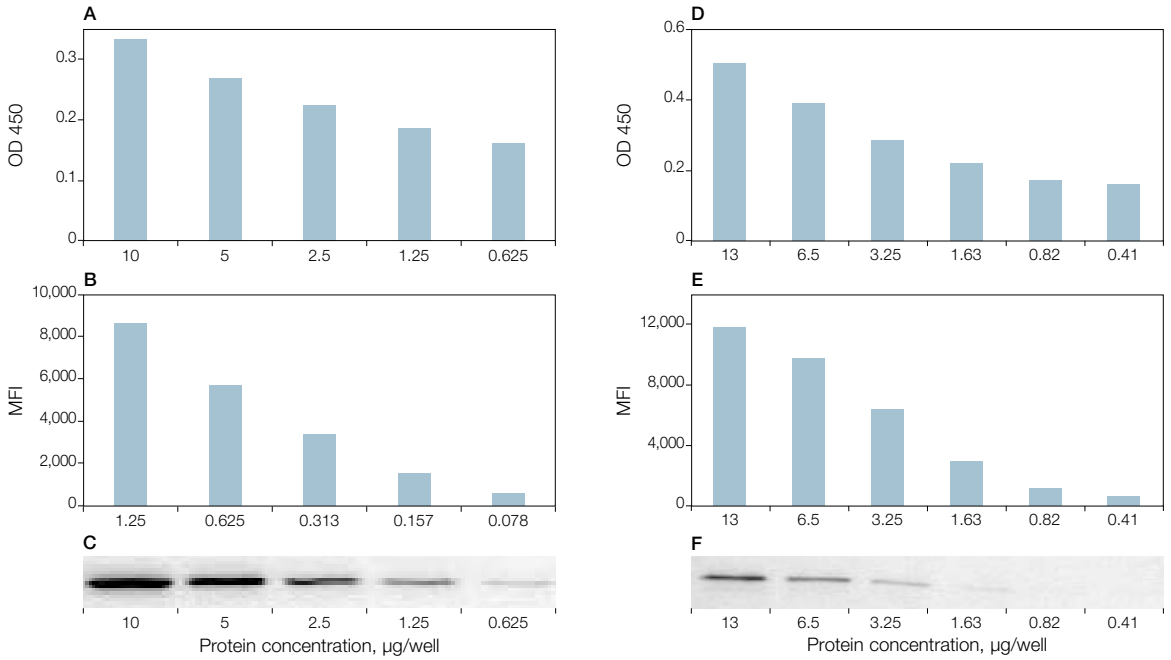


Fig. 3. Total ERK detection. A–C, HEK 293 cell lysate; **D–F**, HeLa cell lysate. **A** and **D**, ELISAs; **B** and **E**, Bio-Plex assays; **C** and **F**, western blots.

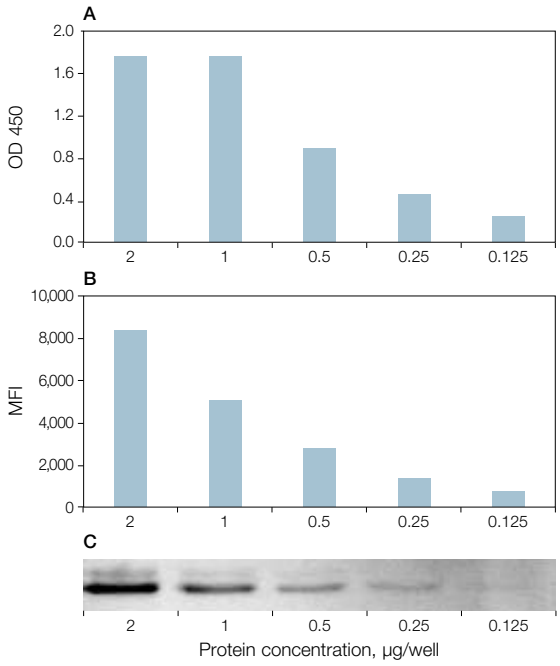


Fig. 4. Phospho-ERK detection. Samples were EGF-treated HEK 293 cell lysates. **A**, ELISA; **B**, Bio-Plex assay; **C**, western blot.

Conclusions

- For the detection of total p38 MAPK in HEK 293 cells, both the Bio-Plex assay and ELISA correlated well with the western blot. The Bio-Plex assay showed saturation at the high end but potential to detect the target in further-diluted samples. With the HeLa lysate, the Bio-Plex assay detected the target with good sensitivity and demonstrated a broad detection range that correlated well with the western blot bands. The target was not detectable by ELISA
- For the detection of phospho-p38 MAPK in HEK 293 cells, the Bio-Plex assay demonstrated a broad detection range at the high end and good correlation with the western blot. Bio-Plex and ELISA assays both showed potential for higher sensitivity toward more dilute samples
- For the detection of total ERK, the Bio-Plex assay demonstrated better signal distinction and sensitivity compared to ELISA
- For phospho-ERK detection, the ELISA showed saturation at the high end. The Bio-Plex assay showed better signal distinction, dose response, and correlation with western blots compared to ELISA

Information in this tech note was current as of the date of writing (2004) and not necessarily the date this version (Rev A, 2007) was published.

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