Cell Biosciences™

Characterization of phosphoprotein signaling in limited biological samples using the Cell Biosciences CB1000

The Cell Biosciences CB1000 system enables detailed characterization of phosphoprotein signaling processes in extremely small biological samples. Whereas traditional protein analysis techniques require thousands to millions of cells, the CB1000 requires as few as 25 cells per assay.

Protein phosphorylation has a central role in the regulation and the deregulation of many cellular processes. A variety of techniques have been developed to characterize protein phosphorylation events in cellular and tissue samples, including western blotting, immunoassays in various formats and specialized mass spectrometry protocols. These traditional techniques generally are hampered by the need for large amounts of starting material—in many cases, millions of cells. The Cell Biosciences CB1000 system (**Fig. 1**) was developed to enable protein phosphorylation studies in biological samples too small for traditional protein characterization techniques. Limited samples such as primary cells, rare cell populations and microdissected tissue sections can be analyzed using straightforward protocols.

The CB1000 system is an automated, capillary-based immunoassay platform[1]. As in western blot analysis, proteins from complex biological samples are separated, immobilized and probed with specific antibodies. However, the CB1000 system uses a capillary isoelectric focusing (cIEF) separation to resolve the various phosphorylation states of signaling proteins (**Fig. 2**). The capillary format provides rapid, high-resolution separations, with a typical separation time of 20 minutes. After separation, proteins are chemically linked to the capillary wall. This linkage step is achieved through UV-light activation of a proprietary photoactive capture chemistry coating on the inner surface of each separation capillary. Proteins immobilized on the capillary wall then are washed and probed with primary and secondary antibodies. Secondary antibodies are conjugated to horseradish peroxidase, which enables ultrasensitive chemiluminescence detection. After addition of the detection reagents luminol and hydrogen peroxide, chemiluminescence signal is collected through the capillary wall.

A characteristic separation of the phosphorylated isoforms of human ERK1 and ERK2 in HT-29 human colorectal cells is shown in **Figure 3**. ERK1 and ERK2 both are believed to be activated by dual threonine/tyrosine phosphorylation, at Thr202 and Tyr204 for human ERK1, and at Thr185 and Tyr187 for human ERK2. The CB1000 separation revealed six protein bands, representing the non-, mono- and dually phosphorylated forms of ERK1 and ERK2. Because human ERK1 and ERK2 are distinct but similar proteins, the isoforms of both proteins are recognized by a single, pan-ERK antibody. The single-antibody assay format is exceptionally useful for estimating quantitative distributions of phosphorylation. For example, from the data (**Fig. 3**), we estimated that 64% of ERK2 was present as the nonphosphorylated state, 12% as monophosphorylated and 24% as dually phosphorylated. The CB1000 Compass™ software represents separation data as a trace of peaks or as a virtual gel image (**Fig. 3**).

Although the CB1000's cIEF assay format is a relatively recent development, the technique has been used to study a wide variety of

Figure 1 | The Cell Biosciences CB1000 system measures protein phosphorylation events and other post-translational modifications using capillary-based nanofluidic immunoassays.

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APPLICATION NOTES

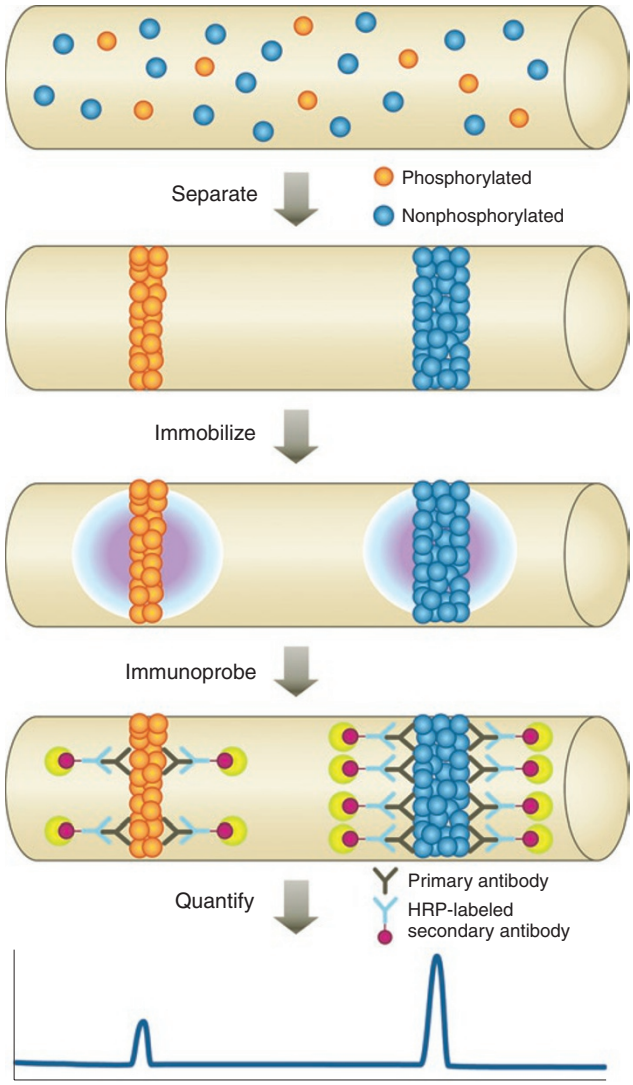

Figure 2 | CB1000 cIEF assay format. The analytical process can be viewed as a sequence of five steps. First, the capillary is loaded with a 400-nanoliter mixture of lysate, fluorescently labeled pI standards and ampholytes. Second, voltage is applied across the capillary to drive the cIEF separation, and individual proteins and pI standards concentrate at their isoelectric points. Third, the capillary is exposed to UV light, activating the linking chemistry and locking the separated protein isoforms to the capillary wall. Fourth, the capillary is rinsed and immunoprobed for specific proteins. Luminol and hydrogen peroxide are added to catalyze the chemiluminescent generation of light, which is captured by a charge-coupled device (CCD) camera. Fifth, the digital image is analyzed and quantitative results are presented by the Compass software. HRP, horseradish peroxidase.

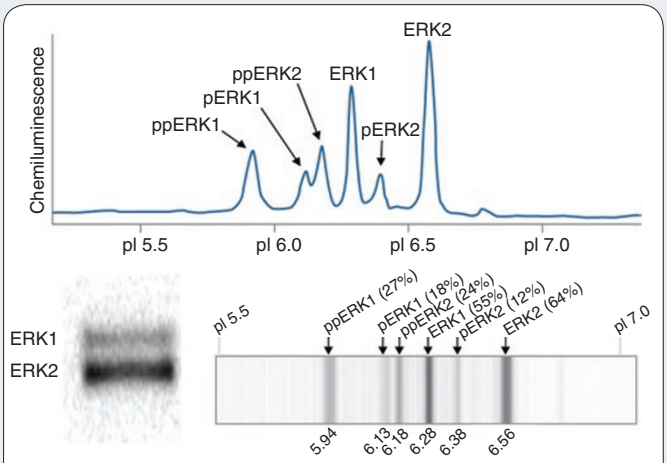

Figure 3 | Analysis of ERK1 and ERK2 in HT-29 human colorectal cells. Conventional western blot results are variable, semiquantitative and provide limited isoforms identification (left). In contrast, the CB1000 provides detailed phosphoisoform profiles (right), as a trace (top) or a virtual gel image (bottom).

samples, including cultured mammalian cells, sorted hematopoietic cells, tumor xenografts, human tumor biopsies, fine needle tumor aspirates and laser-capture microdissections. A particularly noteworthy study appeared recently in *Nature Medicine*[2]. In this work, the authors used Cell Biosciences cIEF assays to quantify levels of MYC oncoprotein and B-cell lymphoma protein-2 (BCL2) in lymphoma samples and to characterize the effects of imatinib treatment on the activation of ERK1/2, MEK1, STAT3, STAT5, JNK and caspase 3 in chronic myelogenous leukemia cells. The authors report using only 4 nanoliters of tissue lysate per assay.

In summary, the Cell Biosciences CB1000 system provides a unique view of cell signaling processes in extremely small biological samples. CB1000 assays are being used to uncover fundamental mechanisms controlling cell proliferation and cell death, to accelerate the development of kinase inhibitor therapeutics and to help identify new prognostic and diagnostic disease biomarkers.

1. O'Neill, R. *et al.* Isoelectric focusing technology quantifies protein signaling in 25 cells. *Proc. Natl. Acad. Sci. USA* **103**, 16153–16158 (2006).
2. Fan, A.C. *et al.* Nanofluidic proteomic assay for serial analysis of oncoprotein activation in clinical specimens. *Nat. Med.* **15**, 566–571 (2009).