USING ELISA IN SITU FOR THE SENSITIVE DETECTION OF NEUROTROPHIC FACTOR RELEASE

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This article reviews three papers in which the highly sensitive ELISA in situ assay method was used to detect low levels of neurotrophic factor release in cells. In all three studies, the authors used Promega's BDNF and NT-3 E_{max}® ImmunoAssay Systems.

Introduction

The search for faster and more sensitive ELISA assays for detecting neurotrophic factor (NF) release in neuronal cultures has traditionally been an important focus for researchers in the field of neuroscience. NFs play an essential role in a number of processes in the central and peripheral nervous systems including the maintenance of hippocampal synaptic plasticity, the regulation of synaptic efficacy and the perception of noxious stimuli. Balkowiec and Katz (1) developed an ELISA protocol—termed ELISA in situ—in which cells are grown in plates precoated with an NF-specific antibody. This in situ format, when compared with the conventional sandwich ELISA protocol, provides a vastly increased level of sensitivity for detecting NF release into the extracellular milieu. Indeed, using the conventional protocol, Balkowiec and Katz (1) were unable to detect NFs in primary sensory neuron cultures previously shown to have an increased NF content with the in situ assay. They concluded that the improved sensitivity of the ELISA in situ assay is a direct result of the immediate capture of the NFs as they are released from the cell (Figure 1). As discussed below, other groups have since incorporated this 'higher sensitivity' ELISA format into their own studies of the precise roles that NFs play in signal transduction and regulation of gene expression.

This in situ format provides a vastly increased level of sensitivity.

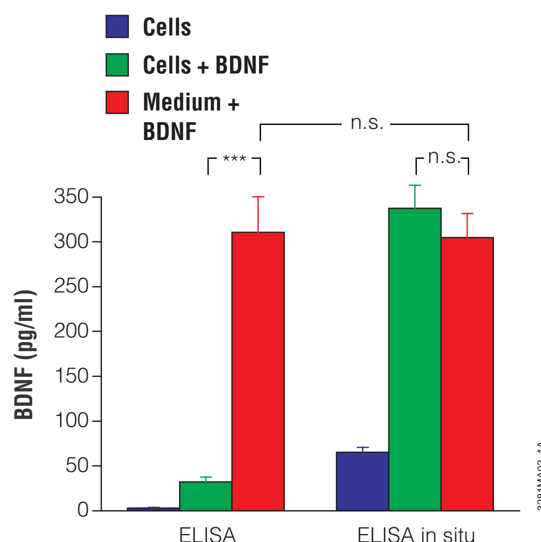

Figure 1. Detectability of exogenous BDNF by standard ELISA versus ELISA in situ. BDNF (Cat.# G1491; 500pg/ml) was added at plating to culture medium alone and to NPG cultures and incubated in the absence (standard ELISA) or presence of Anti-BDNF monoclonal capture antibody (ELISA in situ). BDNF levels were also measured in control cultures to which exogenous BDNF was not added. ***P<0.001; n.s., not significant. Reprinted from *Neural Notes* Issue 19.

Using ELISA in situ for Measuring BDNF Release Following Nociception

BDNF is one of the NFs involved in the noxious stimulation response (nociception) pathway. Its synthesis is carried out at the endings of primary sensory neurons or, as they are more commonly known, primary afferents. Signal transmission across the primary/secondary neuron synapses has been well documented in the literature, and nerve fibers are known to play an essential role in this process. Lever *et al.* (2) used the ELISA in situ assay to evaluate the amount of BDNF released into these synapses following electrical and chemical stimulation of dorsal roots. A number of techniques and agonistic/antagonistic compounds were used, and the results confirmed the roles of BDNF in synaptic signaling. For example, using high-frequency stimulation of primary neuron fibers, they observed BDNF release into the synaptic junction. Moreover, tetrodotoxin (TTX), a drug that is involved in the blockade of action potentials, abolished this electrical stimulation, confirming that BDNF release really was consequential to the conduction of electrical impulses. Further studies using the novel ELISA in situ assay have not only demonstrated a possible retrograde induction of BDNF by NGF but have also elucidated the mechanism by which noxious substances such as capsaicin trigger a stimulus response involving BDNF.

ELISA in situ and Neurotrophin Induction

Recent studies using ELISA in situ have also revealed much about the mechanisms through which neurotrophins exert their effect on target cells. As a result, we now know that neurotrophin upregulation of differentiation and survival of neurons is mediated through the activation of Voltage-Gated Calcium Channels (VGCC). In a set of experiments using hippocampal cultures and antibodies directed

against the neurotrophin NT-3, Boukhaddaoui *et al.* (3) demonstrated that in vitro VGCC activation involves the upregulation of NT-3/TrkC signaling, which subsequently leads to a calbindin-D28k phenotype. Indeed the treatment of these cells with either the anti-NT-3 or anti-TrkC antibodies significantly reduced the number of calbindin-D28k-positive neurons. These in vitro results corresponded with subsequent in vivo studies using $NT\text{-}3^{-/-}$ knockout mice that exploited the improved sensitivity of the ELISA in situ assay to demonstrate a close coupling between VGCC and NT-3 activation in the development of calbindin-D28k-positive neurons. Results with the in situ assay further demonstrated how VGCC inhibitors could induce an overall reduction in extracellular NT-3 levels. The next review highlights the use of ELISA in situ assays to determine how both NT-3 and BDNF regulate expression of particular phenotypes within cortical neurons and how such regulation occurs in a spatial-temporal manner.

Investigating Spatial-Temporal Expression of Neuronal Molecular Markers

The development of a functionally mature cortex involves complex cell-to-cell and cell-to-environment interactions. While it is clear that the various phenotypic features that define each cell type accumulate at different stages of cortex development, the specification of many of these different features occurs at well-defined stages of the developmental process. In contrast, some phenotypic features appear to be dependent on the surrounding environment, and consequently, their expression is more dynamic. An example of changing expression is seen in the neuropeptides and calcium binding proteins whose expression is strongly influenced by changes in expression of transcription factors. For example, the spatial-temporal expression of the *vgf* gene, which encodes the VGF neuronal polypeptide, is only observed in select regions of the cortex during fetal development and the early postnatal period. However, after two weeks following birth, VGF expression is observed in almost all regions of the cortex. Eagleson *et al.* (4) carried out extensive studies aimed at identifying the cellular and molecular mechanisms that 'trigger' the expression of VGF in the developing cortex. Using the ELISA in situ assay, these authors measured the level of secretion of BDNF and NT-3 from specific regions of cortical tissue, more specifically the perirhinal and the occipital neurons. This led them to conclude that a localized accumulation of these neurotrophins, rather than an overall distribution across the cortical tissue, was responsible for the increased levels of VGF expression. As the authors point out, a standard ELISA would not have been sensitive enough for this kind of region-specific measurement given that the standard assay would measure total levels of neurotrophin across the whole cortical tissue.

Conclusion

ELISA in situ is a highly sensitive method for measuring low levels of neurotrophin and NF secretion in which cells are cultured directly on a plate precoated with antibody. Originally developed by Balkowiec and Katz (1), we have reviewed here three papers where the in situ assay has been used for studies in synaptic signaling, neurotrophin-induced differentiation and cortical development. In all three studies, the authors used Promega's BDNF and NT-3 E_{max}® ELISA Immunoassay Systems for setting up the increased-sensitivity ELISA in situ protocol.

References

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Ordering Information

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