Available online at www.sciencedirect.com
SCIENCE DIRECT®
J. Biochem. Biophys. Methods 65 (2005) 20–29
JOURNAL OF biochemical and biophysical methods
www.elsevier.com/locate/jbbm

A simplified probe preparation for ELISA-based NF-κB activity assay

Si Jin [a], Deqin Lu [a], Shiqiao Ye [a], Hong Ye [a], Liping Zhu [a], Zuohua Feng [c], Shengyuan Liu [a], Dixun Wang [a], Qinghua Hu [b,*]

[a] *Department of Pathophysiology, Tongji Medical College, Huazhong University of Science and Technology; Key Laboratory of Pulmonary disease of Ministry of Health of China, Wuhan 430030, P.R. China*
[b] *Department of Medicine, Division of Cardiology, Johns Hopkins Bayview Medical Center, Asthma and Allergy Center, Room LA2, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-2780, USA*
[c] *Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, P.R. China*

Received 16 March 2005; received in revised form 26 August 2005; accepted 31 August 2005

Abstract

Nuclear factor-κB (NF-κB) is critically involved in the transcriptional regulation of many genes and multiple biological and pathobiological processes. To efficiently monitor and to rapidly screen NF-κB transcriptional activity, an ELISA-based assay has been increasingly and successfully employed as a new method in a variety of cell lines and experimental models since its first demonstration and recent development. In the ELISA-based assay, NF-κB is captured by a double-stranded DNA probe pre-linked on multi-well plates. Typically, the DNA probe contains the double-stranded consensus binding sequence for active NF-κB and another double-stranded sequence linking the consensus binding sequence with the plate (linker sequence). Since nuclear factor has no binding activity with single-stranded DNA, we modified the probe construction as containing the double-stranded consensus binding sequence and a single-stranded-linker sequence. Our results show that this kind of probe is highly sensitive and specific for NF-κB activity assay, whereas the preparation of this kind of probe is much more convenient. A single-stranded-linker sequence may largely decrease nonspecific protein binding and thus increase the sensitivity of this assay.

Keywords: Nuclear factor-κB; ELISA; Oligonucleotide probe

* Corresponding author. Tel.: +1 410 550 3378; fax: +1 410 550 2090.
E-mail address: qinghuaa@jhmi.edu (Q. Hu).

0165-022X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbbm.2005.08.006

1. Introduction

Several traditional techniques have been successfully established to reveal the critical role of nuclear factor-κB (NF-κB) in a variety of cellular responses or biological processes [1–3] such as our previous study on Ca^{2+} oscillation frequency-regulated NF-κB transcriptional activation [2]. These include electrophoretic mobility shift assay (EMSA), also called gel retardation [4]; reporter gene assays like chloramphenicol acetyltransferase (CAT) [2], luciferase [5] and green fluorescent protein [6]; cytoplasmic/nuclear distribution of NF-κB and/or its inhibitory protein, I-κB revealed by Western blot or immunofluorescent staining [7]; as well as the specific recognition by antibodies of the nuclear localization sequence (NLS) of NF-κB [8]. Unfortunately, all these methods have major disadvantages, such as being highly time-consuming, requiring radioactive labeling, constituting an indirect assay, or producing non-quantitative measurements.

An ELISA-based nuclear factor activity assay was originally described by Gubler and Abarzua [9] and has been recently modified by Renard et al. [10]. In this kind of assay, the nuclear factor of interest is captured by a double-stranded oligonucleotidic probe containing the consensus binding sequence and then detected by a primary antibody, followed by a secondary antibody conjugated to horseradish peroxidase. This method is convenient, sensitive and quantitative. In fact, this kind of assay has been increasingly and extensively employed in a variety of experimental research projects [11–18].

A key step in this kind of assay is the probe design and preparation. As illustrated in Fig. 1, the regular probe usually contains a double-stranded consensus binding sequence and a double-stranded-linker sequence. The linker sequence is often biotin-labeled to allow it to be fixed on an avidin-coated well. In the probe design, the consensus binding sequence serves as a binding site for specific capture of NFκB. The linker sequence between avidin and the consensus binding sequence is also necessary, presumably providing sufficient space for the binding of NFκB to the consensus binding sequence. However, the preparation of the probe is still dependent on PCR and subsequent purification of the PCR products [10] with the potential problems of contamination or lack of specificity.

In the present study, we propose a new design of the probe based on a structure using the double-stranded consensus binding sequence with a single-stranded-linker sequence. The probe preparation becomes much more convenient by eliminating the process of PCR and its subsequent product purification. In addition, this kind of probe should theoretically increase the sensitivity of the assay since a protein like NFκB has no binding activity with single-stranded DNA. Our results show that this new probe is highly sensitive, specific and reliable in detecting NFκB transcriptional activity.

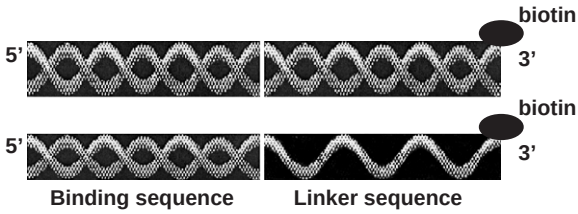

Fig. 1. Schematic illustration of probe design for ELISA-based transcription factor activity assay (upper: classical; lower: modified).