ELSEVIER

Journal of Immunological Methods 323 (2007) 132–138

JIM
Journal of Immunological Methods

www.elsevier.com/locate/jim

Research paper

Development of a modified microtiter plate with a concave portion in the center

Kimio Katsuta [a,*], Hideo Namiki [b], Kouji Matsushima [c]

[a] *Bioarc Laboratories, Saichi-Yoyogi Bldg. 4F, 5-15-9, Sendagaya, Shibuya-ku, Tokyo 151-0051, Japan*
[b] *Integrative Bioscience and Bioengineering, Graduate School of Science and Engineering, Waseda University, Shinjukuku, Tokyo 169-0051, Japan*
[c] *Department of Molecular Preventive Medicine, School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

Received 16 December 2006; received in revised form 7 April 2007; accepted 12 April 2007
Available online 15 May 2007

Abstract

We have developed a modified microtiter plate which has following advantageous features and functions to both conventional microtiter plate and protein array, such as 1) use of conventional microtiter plate reader and washer, and 2) allowance of simultaneous reaction in the same liquid for all wells. Four proteins of human serum albumin, human C-reactive protein (CRP), human plasminogen and human MIP-1α as sample proteins, were measured with the modified microtiter plate. Although the reaction liquid of each wells on the modified microtiter plate shares through concave portion, its antigen/antibody in each well is independent. The independence of the reaction is supported by the result that the above four proteins produced dose–response curves simultaneously. Unlike a conventional protein array, our plate does not need the drying process for antibody adhesion to the plate, preventing inactivation of the antibody. And one can detect the antigen/antibody reaction using the enzymatic amplification reaction (for example utilizing the biotin–streptavidine interaction) like a conventional plate. In addition to these features, our microtiter plate also has the merit of eliminating the so-called "edge effect".

Keywords: Protein array; Microtiter plate; ELISA; Concave portion

1. Introduction

At present, many researchers are trying to develop a complete protein array for simultaneous analysis of proteins. All researchers have been applying technology of gene chip to protein array. They have been trying to produce a small spot. The objectives of such microarrays are 1) to quantify many species of proteins at a time in the same liquid layer, showing possibility of comprehensive analysis, 2) using a small quantity of sample, such as one drop of blood, 3) in a short time, usually in 1 h. Attempts to develop protein arrays aiming at these three objectives are going on in over several hundreds of laboratories (For examples; Alan, 2004; Angenendt et al., 2002; Beath, 2002; Boutell, 2004; Bussow et al., 2001; Dunham, 2001; Ham, 2001; Michaud, 2003; Peters, 2005; Uetz et al., 2000; Washburn, 2003; Washburn et al., 2001; Wildt et al., 2000 and Willis, 2003).

However in the assay development of protein arrays, two problems remain to be solved. After protein adsorption

Abbreviations: ELISA, Enzyme-Linked Immunosorbent Assay; CRP, C-reactive protein; PBS, Phosphate buffered saline; TMB, Tetramethylbenzidine; SD, standard deviation.

* Corresponding author. Tel.: +81 3 5312 2920; fax: +81 3 5312 2965.
E-mail address: Katsuta@bioarc.co.jp (K. Katsuta).

0022-1759/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2007.04.007

on the array surface, the protein molecules may lose over 90% of their activity upon drying. In addition, one cannot use an enzymatic amplification reaction, as it is not possible to discriminate a defined protein spot on the array.

Does a protein array have to be as small as a gene chip? If a microtiter plate employed for standard ELISA could be used, it would also be possible to use a conventional plate reader and plate washer. The achievement of independent antigen–antibody reaction of various kinds of protein in the whole same fluid phase and the detection of each reaction of the next can express "to quantify many species of proteins at a time in the same liquid layer". Hence, no special device would be needed. Since different proteins can be determined in each well, it can be possible to determine simultaneously, precisely and reproducibly 96 species of proteins in a 96-well plate. However, in view of the structure of a conventional 96-well microtiter plate, simultaneous determination in the same liquid layer is impossible.

We investigated whether conventional microtiter plates could be modified, and whether such modified microtiter plate can enables independent antibody-based detection of distinct proteins within each well, simultaneously.

2. Materials and methods

2.1. Materials

Human albumin affinity purified was obtained from Biogenesis (Poole, U.K). Human C-reactive Protein (CRP) was from CHEMICON International (Temecula, CA USA). Human Glu-plasminogen was from Haematologic Technologies Inc. (Essex Junction, VT USA). Human macrophage inflammatory protein-1 alpha (human MIP-

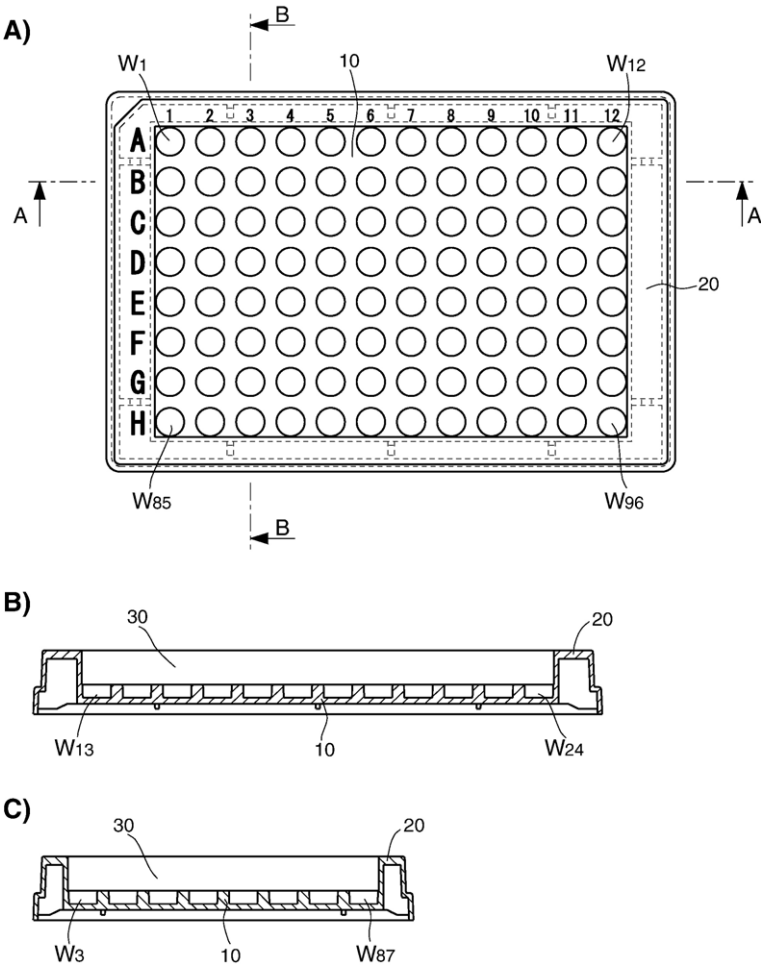

Fig. 1. General view of modified microtiter plate. General view of modified microtiter plate with Cross section at A-A of plane view, and with Cross section at B-B of plane. 10; Bottom, 20; Sidewall part, 30; Concave portion, W; refers to each well and the number at the bottom on the left side refers to the corresponding well No.