cell signaling

Application Notes

Panorama™ Mouse/Rat Tissue Extract Protein Array Kit: A New Tool for Protein Expression Analysis

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- Simultaneously study protein expression in different tissue and cell fraction extracts
- Rapidly perform profiling assays, can be completed in ~5 hours
- High density spots ensure strong signals and greater sensitivity
- Low background staining and long shelf life due to proprietary slide treatment

Introduction

Today, global molecular characterizations of complex biological samples are increasingly used. Therefore, methods for simultaneous expression analysis of proteins in different tissues, i.e. brain, liver, spleen, and cerebrum, as well as different localizations within the cell, e.g. cytoplasm and nucleus, are required. Tissue extract protein arrays provide a convenient and straightforward solution for profiling of protein expression in different species' tissue protein samples in a miniaturized "dot-blot" like method. This approach is rapid and only requires specific antibodies.

Mouse/rat tissue extract protein content

Sigma's new Panorama™ Mouse/Rat Tissue Extract Protein Array Kit (Product Code MRPA1) is the first in a line of protein extract-based microarrays. The array contains 62 extracts from 10 mouse and 12 rat tissues. Each tissue is represented by 3 extracts, derived from the total tissue as well as its nuclear and cytoplasmic cell fractions. All extracts were prepared in denaturing, reducing buffer and boiled. Extracts of the same origin, i.e. total, cytoplasm, and nucleus, from different tissues are spotted at equal protein concentration depending on the origin. In addition, a series of positive and negative controls (IgGs from goat, rabbit, rat and mouse; KLH and BSA) are spotted. Information on specific positioning of extracts and controls can be found in Figure 1 and Table 1 (p. 15).

Array format

The protein extracts are spotted on nitrocellulose-coated glass slides. The three-dimensional structure of the nitrocellulose layer allows for a high protein binding capacity, a large dynamic range and reproducibility. Each tissue is printed in a separate grid (Figure 1). Each grid contains total, cytoplasmic and nuclear protein extracts from mouse or rat tissue. Each extract is spotted in three protein concentrations. IgGs from different species (goat, rabbit, rat and mouse) are spotted as positive controls. Overall, the array contains a total of 273 spots (Figure 1).

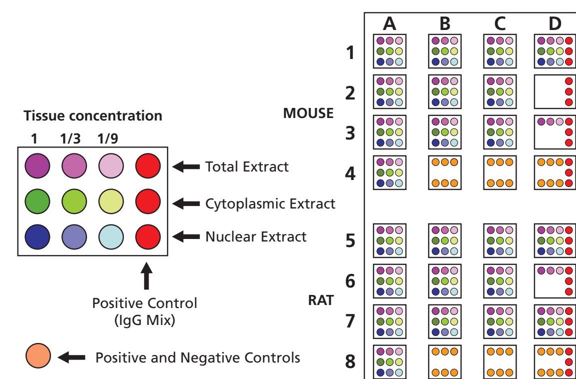

Figure 1. Microarray format.

Detection method

The Panorama™ Mouse/Rat Tissue Extract Protein Array Kit can be used for parallel protein expression analysis in various tissue extracts with antibodies. In order to enable this each spotted extract is pre-tested for its ability to bind antibodies in the array using several antibodies. After probing the array with an antibody and subsequent washing to remove unbound antibodies, bound antibodies can be detected using either fluorescence or chemiluminescence. Four labeling systems are applicable for readout: the antibody itself is conjugated either with a (i) fluorophore or with (ii) Peroxidase enzyme; alternatively the bound antibody is detected by a secondary antibody, which is conjugated with either a (iii) fluorophore or (iv) Peroxidase enzyme for chemiluminescence generation.

The slides are read for differential expression using standard microarray scanning instrumentation when a fluorescent labeling system is applied. In the case of chemiluminescent detection regular chemiluminescent developers can be used, and possibly CCD-camera based reader with sufficient resolution for proper quantitation (not tested). The total assay takes about 5 to 6 hours. For details of the protocol please refer to the technical bulletin.

Verification of tissue fractionated extracts (nuclear versus cytoplasmic)

Tissue extracts were prepared and then separated to cytoplasmic and nuclear fractions. Before printing the different tissue extracts onto the slides, the cytoplasmic and nuclear fractions of all extracts were validated by using various antibodies specific for nuclear or cytoplasmic proteins. For example, Figure 2A represents the results obtained with rat heart extracts probed with monoclonal antibody to catalase (Product Code C 0979), a protein that is mainly present in the cell cytoplasm. Indeed, only the total and cytoplasmic fractions revealed the presence of catalase. When mouse kidney extracts were tested with rabbit polyclonal antibodies to calbindin (Product Code C 2724, a cell cytoplasmic marker) and to HDAC1 (Product Code H 3284, a nuclear marker), the fractionated extracts were clearly defined (Figure 2B).

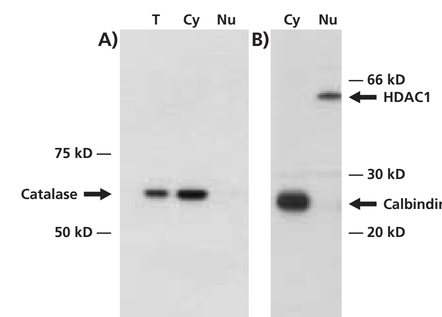

Figure 2. Equal amounts of rat heart (A) or mouse kidney (B) protein extracts were separated by SDS-PAGE and tested by immunoblotting with either (A) monoclonal anti-Catalase (Product Code C 0979) or with (B) rabbit anti-HDAC1 (Product Code H 3284) and rabbit anti-Calbindin (Product Code C 2724).
T = Total extract
Cy = Cytoplasmic fraction
Nu = Nuclear fraction

Differential expression of proteins in tissues

The Panorama™ Mouse/Rat Tissue Extract Protein Array Kit was used to profile the expression of proteins in mouse and rat tissues using antibodies to several protein targets. A labeling system with secondary antibody conjugated to Peroxidase and CPS1 chemiluminescent substrate was used. Resulting chemiluminescence was detected using autoradiograph film. As expected, each antibody showed a different pattern of protein expression (Figure 3). The results clearly show that some of the proteins are broadly expressed in many tissues (e.g., vinculin), while others are more specific to certain tissues (e.g., neurofilament).

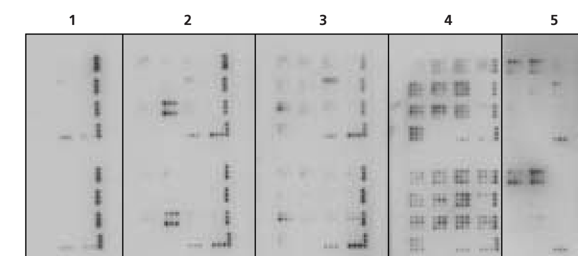

Figure 3. Rabbit primary antibodies to different protein targets were tested, each on a separate slide:
1. Negative control-only secondary antibody: anti-rabbit IgG, Peroxidase conjugate
2. Anti-Androgen Receptor (Product Code A 9853)
3. Anti-Cofilin (Product Code C 8736)
4. Anti-Vinculin (Product Code V 4139)
5. Anti-Neurofilament 200 (Product Code N 4142)
The antibodies were developed with anti-rabbit IgG, Peroxidase conjugate and a chemiluminescent substrate. Note the different protein profile expression of the proteins.

Comparison of fluorescent and chemiluminescent detection systems

Two detection systems, fluorescent and chemiluminescent, known from immunoblotting or from DNA and antibody arrays, were tested and compared for sensitivity. A rabbit polyclonal antibody to Neurofilament protein was used in both assays; however, one slide was further incubated with Goat anti Rabbit IgG conjugated to Cy3 and the other slide with the same secondary antibody conjugated to Peroxidase. Very similar results were obtained, leading to the conclusion that under the chosen conditions both methods are comparable (Figure 4).

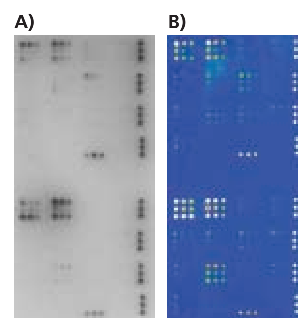

Figure 4. Comparison of fluorescent and chemiluminescent labeling and detection methods. *Anti-neurofilament 200 (Product Code N 4142) was applied on two slides. Slide A was further incubated with a Peroxidase conjugated secondary antibody and developed with a chemiluminescent substrate (CPS1), while slide B was incubated with a Cy3 dye conjugated secondary antibody. Note that similar results are obtained by the two detection methods.*

Summary

We have described the properties of the novel protein extract array system Panorama™ Mouse/Rat Tissue Extract Protein Array Kit. The array contains 62 protein extracts spotted on nitrocellulose-coated slides. The protein extracts are from mouse and rat tissues, subdivided into total, cytoplasmic and nuclear extracts. Different labeling and detection methods have been applied to the Panorama™ Mouse/Rat Tissue Extract Protein Array resulting in similar results.

Ordering Information

Product	Description	Unit
MRPA1	Panorama™ Mouse/Rat Tissue Extract Protein Array Kit	1 kit

Table 1. Spotted Features of the PANORAMA™ Mouse/Rat Tissue Extract Protein Array

MOUSE TISSUE EXTRACTS

A	B	C	D	
Brain Total	Brain Total	Heart Total	Kidney Total	IgG Mix
Brain Cytoplasmic	Brain Cytoplasmic	Heart Cytoplasmic	Kidney Cytoplasmic	IgG Mix
Brain Nuclear	Brain Nuclear	Heart Nuclear	Kidney Nuclear	IgG Mix
Liver Total	Lung Total	Skeletal Muscle Total		IgG Mix
Liver Cytoplasmic	Lung Cytoplasmic	Skeletal Muscle Cytoplasmic		IgG Mix
Liver Nuclear	Lung Nuclear	Skeletal Muscle Nuclear		IgG Mix
Spleen Total	Stomach Total	Testis Total	Thymus Total	IgG Mix
Spleen Cytoplasmic	Stomach Cytoplasmic	Testis Cytoplasmic		IgG Mix
Spleen Nuclear	Stomach Nuclear	Testis Nuclear		IgG Mix
Liver Total	Mouse IgG	Goat IgG	BSA	IgG Mix
Liver Cytoplasmic				IgG Mix
Liver Nuclear	Rat IgG	Rabbit IgG	KLH	IgG Mix

RAT TISSUE EXTRACTS

A	B	C	D	
Cerebrum Total	Cerebellum Total	Heart Total	Kidney Total	IgG Mix
Cerebrum Cytoplasmic	Cerebellum Cytoplasmic	Heart Cytoplasmic	Kidney Cytoplasmic	IgG Mix
Cerebrum Nuclear	Cerebellum Nuclear	Heart Nuclear	Kidney Nuclear	IgG Mix
Liver Total	Lung Total	Skeletal Muscle Total	Ovary Total	IgG Mix
Liver Cytoplasmic	Lung Cytoplasmic	Skeletal Muscle Cytoplasmic		IgG Mix
Liver Nuclear	Lung Nuclear	Skeletal Muscle Nuclear		IgG Mix
Spleen Total	Stomach Total	Testis Total	Thymus Total	IgG Mix
Spleen Cytoplasmic	Stomach Cytoplasmic	Testis Cytoplasmic	Thymus Cytoplasmic	IgG Mix
Spleen Nuclear	Stomach Nuclear	Testis Nuclear	Thymus Nuclear	IgG Mix
Liver Total	Mouse IgG	Goat IgG	BSA	IgG Mix
Liver Cytoplasmic				IgG Mix
Liver Nuclear	Rat IgG	Rabbit IgG	KLH	IgG Mix