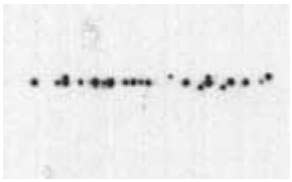
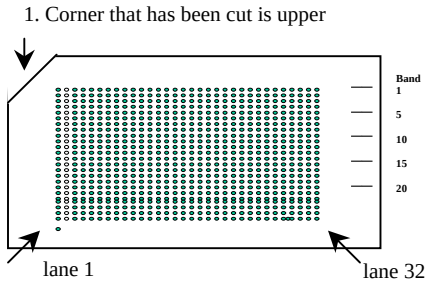
Mega Western Protein Array:

Introduction

Mega Western Protein Array is a novel tool for analysis of protein expressions, combining the unique features of tissue protein micro-arrays and Western blots. Using Mega Western Protein Arrays, not only protein expressions but also protein sizes can be determined in more than 30 different tissues. The dimensions of CytoMol's Mega Western Protein Array are only 1x2 inches, and the detection method is the same as is commonly employed for Western blot analysis.



Actual Size: 1" x 2"



Signal Locator

Human Adult Normal Tissue Mega Western Protein Array Composed of 32 Lanes. *Left:* A Mega Western Protein Array (Cat# PWB8234480) was blotted glyceraldehyde -3-phosphate dehydrogenase (GAPDH) antibody, detected by non-radioactive method, and exposed to X-film for 10 seconds. *Right:* A transparent Signal Locator sheet is included for easy identification of the lanes and sizes of the signals.

Features

- Reproducible results - The product comes in an array format. Any two membranes are nearly identical in compared with the traditional western blot.
- Higher sensitivity - Protein species are highly concentrated per unit area on the membrane and are readily accessible to antibodies
- Versatile - Suitable for both radioactive and non-radioactive detection
- Easy of use - The membrane can be treated/handled the same as a conventional Western blot.
- Economical - One blot analysis reveals protein expressions in more than 30 different tissues.
- Antibody saving - Save at least 4 times of the antibody in compared with using traditional western blot.
- Stable - Dried storage condition at room temperature

Applications

- Identification of tissue-specific protein expression in a wide variety of tissues
- Validating the quality of antibodies in industrial level
- Pattern analysis of protein expression
- Comparison of expression levels of target proteins
- Determination of the size and relative abundance of target proteins in different tissues
- Examination of protein iso-forms due to alternative splicing and premature termination of specific gene transcripts, and post-translational modifications.

Description

CytoMol's Mega Western Protein Array was developed using a patent-pending proprietary technology. The Mega Western Protein Arrays are manufactured using high quality denatured protein lysates from documented tissue sources. A protein sample from each tissue is well electrophoresesed along with a Protein molecular weight marker on a large size SDS-PAGE gel

and eluted into 24 fractions. Each protein fraction covers a defined molecular weight range. The recovered protein fractions were printed on modified nitrocellulose membranes. Each array can contain many more sample lanes than traditional Western Blots, and each lane is composed of 24 fractions. These arrays are suitable for use with both radioactive and non-radioactive methods. A user's manual is included. The Mega Western Protein Array is supplied ready to use, so that you can immediately screen proteins of interest without the hassle of obtaining hard to find tissues and processing the proteins yourself.

Quality Control
The quality of Mega Western Protein Array was verified by blotting against mouse anti-human GAPDH.

Component

Item	Amount	Storage
Mega Western Protein Array	1	4°C
Signal locator	1	Room Temperature
GAPDH antibody	5 µl	4°C

Materials Not Supplied
- Primary antibody
- Secondary antibody
- 1xTTBS
- Blocking Solution
- Substrates for developing
- CCD camera or X ray film developer
- Shaker

Protocol
1. Block the array with 5% (w/v) BSA in 1 x TTBS solution for 1 hour at room temperature with agitation.
2. Make an appropriate dilution of primary antibody in 1 x TTBS. Remove the blocking reagent and add the primary antibody solution. Incubate the array with agitation for one hour at room temperature or overnight at 2-8°C.
3. Wash the array in 1 x TTBS for 5 min, repeat 3-4 times.
4. Incubate the array with the appropriate secondary antibody solution for 1 hour at RT with agitation.
5. Repeat step 3 to remove unbound secondary antibody.
6. Develop the array with proper substrates.
7. Detect the signals with a CCD camera or X ray film.
8. The array can be re-developed if necessary.
11. After stripping, the array can be re-probed.

Re-developing Method
Soak array in 1 x TTBS solution at 2-8°C O/N. On the second day, incubate with secondary antibody and apply wash buffer, add substrates to re-develop.

Stripping Method
1. Use stripping buffer M if the mild condition is sufficient; and use stripping buffer H if more stringent stripping conditions are necessary.
2. Soak the array in stripping buffer and incubate at 50°C for 30 min with occasional agitation (incubate for a longer time or raise temperature to 70°C if the array is not completely stripped).
3. Wash the array twice in a large volume of 1 x TTBS for 10 min at room temperature.
4. Repeat the immunoblotting procedure from the blocking step.

Diagram of Mega Western Protein Array

Mega Western Protein Array is about 1 x 2 inches in size. It contains multiple tissue lysate lanes, and each lane is made up of 24 spots. Each spots within one lane represents a particular range of protein species. The upper left corner is cut to indicate correct orientation.

Results Interpretation

A. Put the Signal Locator transparency sheet on top of the X-ray film to align the signals to the locations of the spots on the membrane. First make sure that the sample lanes are aligned to the signals. Be aware that no signal is obtained in negative control lane. Second, align the marked dots to the dots indicated on the Signal Locator to identify the fraction numbers that contain the signals.

B. Size-estimation of the protein: To estimate the size of the protein after Western analysis, first determine the spot number, which has the strongest signal. There are 24 fractions eluted from equal distance of gel for each tissue arrayed. Each spot contains a range of different sizes of proteins. When spot number is determined, check the following table to estimate the size of the protein.

Spot Number	Sizes (kDa)	Spot Number	Sizes (kDa)
1	213 and up	**13**	46-51
2	161-213	**14**	42-46
3	131-161	**15**	39-42
4	112-131	**16**	35-39
5	98-112	**17**	33-35
6	88-98	**18**	30-33
7	80-88	**19**	28-30
8	74-80	**20**	26-28
9	68-74	**21**	25-26
10	64-68	**22**	23-25
11	57-64	**23**	22-23
12	51-57	**24**	22 and less

C. Multiple Spots for one protein: In conventional Western blot analysis, blotting signal is not always shown as a thin narrow band. Instead, thick band could cover quite some distance. Similarly, for Mega Western Protein Array, it is common that signals for one protein are found in two spots. It is because the protein is at the edge of pre-defined two fractions. Determine fraction with maximal signal strength using the Signal Locator as described above.

Trouble Shooting

1. No signal or weak signal

- The concentration of primary or secondary antibody used was too low
- The blocking buffer used was not correct, and antigen was trapped
- Substrates had lost activity
- Target protein degradation occurred due to improper storage of the array

2. High background

- The concentration of the primary or secondary antibody used was too high
- Insufficient blocking
- Insufficient washing
- The level of Tween-20 in blocking buffer was too low

3. Reverse image on film

- The concentration of the primary or secondary antibody used was too high
- Too much Substrates in the system

Related Products

Western blot, Protein array, Attoglow Western Blot Analysis Kit (pkd71121 or pkd71251), and AttoglowAdvance Western Blot Analysis Kit(pkd73121).

Appendix

Preparation of solutions not supplied with kit:

Solution	Preparation	Stability/temperature	Notes
1 x TTBS	Add 6.05 g Tris base (50 mM), 8.76 g sodium chloride (150 mM) to 800 ml distilled water, adjust pH to 7.5 with HCl; adjusted to 1 liter with distilled water. Add Tween-20 to 0.1% (v/v)	3 months at RT	Do not use sodium azide as an antimicrobial agent as it inhibit HRP
Blocking Solution	Weigh 5 g of BSA and dissolve it in 100 ml 1 x TTBS solution	Freshly made suggested	Can be Stored at 2-8°C over night
Stripping Buffer M	100 mM Glycine, pH 2.7	1 month at RT	Mild stripping buffer
Stripping Buffer H	62.5 mM Tris-HCl, pH 6.7 with 2% SDS and 100 mM 2-Mercaptoethanol	1 month at RT	Harsh stripping buffer

References

1. Ausubel, F. M. , Brent, R., Kingston, R. E, Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K., eds (1994). *Current protocols in Molecular Biology.*Greene Publishing associates and Wiley-Interscience. New York

2. Sambrook, J., Fritsch, E. F. and Maniatis, T. (2001). Molecular Cloning: A Laboratory Manual, 3rd Edition. Cold Spring Harbor Laboratory Press.Plainview, New York.