

Panorama™ Mouse/Rat Tissue Extract Protein Array KitProduct Code **MRPA1**
Storage Temperature 2–8 °C**Product Information****TECHNICAL BULLETIN****Product Description**

The Panorama™ Mouse/Rat Tissue Extract Protein Array Kit is designed for studying protein expression in different tissue extracts. The expression of a protein in an extract is detected using a protein specific antibody. When the antibody binds the protein, this binding is visualized by a sensitive chemiluminescent or fluorescent signal created by directly labeling the primary antibody with a fluorescent dye or peroxidase enzyme, or by the use of a specific secondary antibody conjugated to a fluorescent dye or peroxidase. Each spotted extract is pre-tested for its ability to bind antibodies in the array using several different antibodies. Each extract type is spotted at equal protein concentration depending on its origin i.e. total, cytoplasmic, or nuclear extract. All extracts were prepared in denaturing and reducing buffer and boiled.

Important advantages of the Panorama Mouse/Rat Tissue Extract Protein Array Kit are:

- The profiling assay is fast and can be completed in ~5 hours.
- The extracts are spotted in high density to ensure strong signals.
- A special proprietary treatment of the slides ensures low background staining and long shelf life.

There is a growing need for technologies that allow global molecular characterization of biological samples. The ability to identify a single protein simultaneously in different protein tissue extracts has many applications in basic biological research. A method that can detect targets within a tissue, as well as their differential localization within the cell (cytoplasmic or nuclear) is needed for analysis of known and novel proteins. Tissue extract protein arrays provide one of many solutions that can be used for rapid profiling of protein expression in different tissues of different species in a microarray “dot-blot”-like method.

Important general aspects of the Panorama Mouse/Rat Tissue Extract Protein Array:

- The mouse and rat tissue proteins spotted are extracts of total tissue as well as nuclear and cytoplasmic cell fractions. Each tissue extract and its fractions are spotted in three concentrations. In addition, a series of common positive and negative controls (IgGs from goat, rabbit, mouse and rat; KLH and BSA) are spotted. Information on specific positioning of each extract and control can be found in the Appendix (Figure 1 and Table 1).
- The results obtained using the array are semi-quantitative and should be further evaluated by other methods such as ELISA or immunoblotting assays.
- The slides are sensitive, do not touch the surface of the slides and handle the slides with non-latex, powder-free gloves.
- The slides are recommended for a single use.

Components

Sufficient material is provided for performing 3 array reactions. The buffers are provided in excess.

Panorama Mouse/Rat Tissue Extract Protein Array Slides Product Code T 6450	3 each
QuadriPERM® Cell Culture Vessel Product Code Q 3756	1 each
Tris Buffered Saline, pH 8.0, with TWEEN® 20 Product Code T 9039	1 pouch
Preincubation Buffer Product Code B 7685	20 ml

Reagents and Equipment Required but Not Provided

- Protein specific primary antibody
- Peroxidase labeled or Cy3/Cy5 labeled secondary antibody specific for the species in which the primary antibody was developed
- Non-fat dried milk (NFDM) (Product Code M 7409)
- Enhanced chemiluminescent detection reagent
- Rocking shaker
- Microarray Scanner

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Washing Buffer (TBS-T20) - Open the foil pouch containing the Tris Buffered Saline, pH 8.0, with 0.05 % TWEEN 20 (Product Code T 9039), and dissolve in 1 liter of deionized water. Filter through a 0.45 μm filter.

Array Buffer - Prepare a solution of 0.5 % non-fat dried milk (NFDM) in Washing Buffer.

Storage/Stability

The kit should be stored at 2–8 °C. The kit components are stable for 12 months.

Procedure

Wear disposable gloves (non-latex, powder free) while performing all procedures.

I. Antibody Incubation on the Array

Absolutely do not touch the surface of the array during the procedure. Forceps are recommended.

Allow kit components to reach room temperature before beginning the procedure.

Serum, hybridoma supernatant, ascites fluid, purified antibody, or fractionated sera may be used with this kit. The following are recommendations for initial antibody dilution/concentration for newly tested antibodies:

- Serum, 1:200 dilution
- Hybridoma supernatant, 1:2 dilution
- Ascites fluid, 1:100-1:1,000 dilution
- Purified antibody, 10-20 $\mu\text{g/ml}$
- Fractionated serum, 1:200-1:1,000 dilution

The recommended incubation time for the primary antibody is 2 hours at room temperature; however, when the signal is low, it is recommended to prolong the incubation time to overnight at 2–8 °C. The incubation steps are performed at room temperature on a rocking shaker at a moderate speed (~30 rpm).

It is recommended to use one slide without primary antibody to detect possible non-specific background due to the secondary antibody.

1. Mark each slide using a pencil. Use of a pen or a marker may affect the final results.
2. Add 5 ml of Preincubation Buffer (Product Code B 7685) into a well of the incubation tray (QuadriPERM Cell Culture Vessel) for each slide, and insert the slide with the nitrocellulose side of the slide facing up.
3. Incubate the slide for 10 minutes.
4. Aspirate the Preincubation Buffer from the well of the incubation tray. Wash the slide twice for 1 minute each time with 5 ml of Washing Buffer. Incubate for a further 30-40 minutes in a fresh aliquot of Washing Buffer.
5. In a separate tube, dilute the primary antibody to the appropriate dilution/concentration in 4 ml of Array Buffer. Mix well by inverting the tube (do not vortex).
6. Aspirate the Washing Buffer from the well of the incubation tray. Add the diluted primary antibody to the appropriate slide-containing well. Cover with the lid.
7. Incubate for 2 hours on a rocking shaker with moderate shaking.
8. Wash the slide three times for 10 minutes each time with 4 ml of Washing Buffer.
9. Aspirate all the liquid from the well.
10. If the primary antibody is directly labeled with a fluorescent dye or with peroxidase, proceed to step 14.
11. Dilute the secondary antibody in 4 ml of Array Buffer and add to the well.
12. Incubate for 1 hour in a rocking shaker with moderate shaking.
13. Wash the slide three times for 10 minutes each time with 4 ml of Washing Buffer.
14. When using a fluorescent-labeled antibody, carefully take the slide out of the well and let it dry completely in air for at least 20 minutes (protect from light). For peroxidase-labeled antibodies, develop with enhanced chemiluminescent detection reagent.

II. Scanning the Array

- Scanning can be performed using any microarray scanner. The optimal laser power and PMT should be determined for each scanner individually.
- The slide should be absolutely dry before the scanning. Water may cause background problems.
- Due to the short half-life of the fluorescent dyes, it is recommended that the array be scanned as soon as the experiment is completed and no more than 24-48 hours later.

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Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTION
Weak signal	Low affinity of primary antibody	Increase the incubation time up to 24 hours (step 7).
	Low affinity of secondary antibody	Check laser power and PMT parameters.
High background	Non-specific binding of primary and/or secondary labeled antibody	Decrease the concentration of primary and/or secondary antibody applied on slide.
No signal from a specific antibody	Low level of target protein in the sample	Increase the concentration of primary and/or secondary antibody.
	Recognition of the antigen by the antibody is lost when labeling the antibody (direct assay).	Try an indirect assay using labeled secondary antibody.

Appendix

Figure 1.
Microarray Format

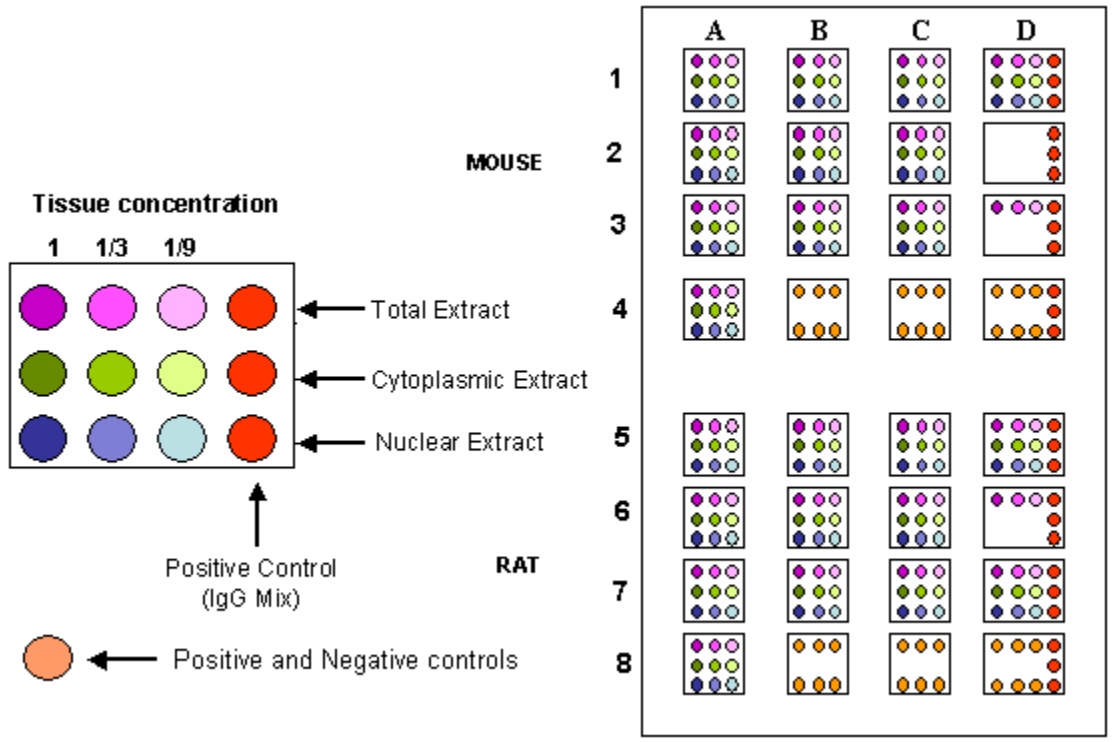


Table 1. Panorama Mouse/Rat Tissue Extract Protein Array Kit

	A	B	C	D	
MOUSE TISSUE EXTRACTS	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	Cerebrum Total	Cerebellum Total	Heart Total	Kidney Total
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

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