Proteome Profiler™ Array

Mouse Apoptosis Array Kit

Catalog Number ARY031

For the parallel determination of the relative levels of mouse apoptosis-related proteins.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Analyzing the expression profiles of apoptosis-related proteins is helpful for understanding the roles these signaling molecules play in mechanisms related to programmed cell death and disease states. The Mouse Apoptosis Array is a rapid, sensitive, and economical tool to simultaneously detect the relative levels of expression of 21 apoptosis-related proteins without performing numerous immunoprecipitations and Western blots. Each capture antibody was carefully selected using cellular extracts prepared from cell lines known to express the target protein.

PRINCIPLE OF THE ASSAY

Capture antibody, negative control, and reference spots have been spotted in duplicate on nitrocellulose membranes. Cell lysates are diluted and incubated overnight with the Proteome Profiler Mouse Apoptosis Array. The membrane is washed to remove unbound material followed by incubation with a cocktail of biotinylated detection antibodies. Streptavidin-HRP and chemiluminescent detection reagents are then applied, and a signal is produced at each capture spot corresponding to the amount of protein bound. Refer to the Appendix for a list and coordinates of analytes and controls.

TECHNICAL HINTS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This kit should not be used beyond the expiration date on the kit label.
- **Do not mix or substitute reagents with those from other lots or sources. Substitution of some high intensity chemiluminescent reagents for Chemi Reagents 1 and 2 may cause either increased background or diminished signal depending on the reagent.**
- Any variation in sample handling, buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- The array membranes are validated for single use only.
- Always use gloved hands and flat-tipped tweezers to handle the membranes.
- Pick up the membranes from the edge on the side with the identification number avoiding the area with the printed antibodies.
- A thorough and consistent wash technique is essential for proper assay performance. Individual arrays should be washed in separate containers to minimize background. Wash Buffer should be removed completely from the membrane before proceeding to the next step.
- Do not allow the membrane to dry out. This will cause high background.
- Avoid microbial contamination of reagents and buffers.
- Other proteins present in biological samples do not necessarily interfere with the measurement of analytes in samples. Until these proteins have been tested with the Proteome Profiler Array kit, the possibility of interference cannot be excluded.
- For a procedure demonstration video, please visit: www.RnDSystems.com/ProteomeProfilerVideo.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Apoptosis Array	889020	8 nitrocellulose membranes each containing 21 different capture antibodies printed in duplicate.	Return unused membranes to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 3 months at 2-8 °C.*
Array Buffer 1	895477	2 vials (21 mL/vial) of a buffered protein base with preservatives.	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 8	895050	21 mL of a buffered protein base with preservatives.	
Lysis Buffer 17	895943	21 mL of a non-denaturing buffered solution with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Detection Antibody Cocktail, Mouse Apoptosis Array	889021	1 vial of biotinylated antibody cocktail; lyophilized.	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	1 vial (2.5 mL)	
Chemi Reagent 2	894288	1 vial (2.5 mL)	
8-Well Multi-dish	607591	Clear 8-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	608136	1 transparency overlay template for coordinate reference.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips
- Gloves
- Deionized or distilled water
- Rocking platform shaker
- Microcentrifuge
- Plastic containers with the capacity to hold 50 mL (for washing the arrays)
- Plastic transparent sheet protector (trimmed to 10 cm x 12 cm and open on three sides)
- Plastic wrap
- Paper towels
- Absorbent lab wipes (KimWipes® or equivalent)
- Autoradiography cassette
- Film developer
- X-ray film (Kodak® BioMax™ Light-1, Catalog # 1788207) or equivalent
- Flat-tipped tweezers
- Flatbed scanner with transparency adapter capable of transmission mode
- Computer capable of running image analysis software and Microsoft® Excel

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Phosphate-Buffered Saline (PBS)
- Lysis buffer 17 (1% Igepal CA-630, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2 mM EDTA, 200 mM Sodium orthovanadate, 5mM NaF)
- Aprotinin (Tocris™, Catalog # 4139)
- Leupeptin (Tocris, Catalog # 1167)
- Pepstatin (Tocris, Catalog # 1190)

PRECAUTIONS

Chemi Reagents 1 and 2 contain Boric Acid which is suspected of damaging fertility or the unborn child.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Since the Mouse Apoptosis Array detects relative expression levels of individual analytes, it is important to include appropriate control samples.

Note: *Sample amount may be empirically adjusted to attain optimal sensitivity with minimal background. Suggested starting ranges are: 200-400 µg for cell lysates samples.*

Cell Lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding Lysis Buffer 17 supplemented with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin. Solubilize cells at 1 x 10^7 cells/mL in this buffer. Pipette up and down to resuspend and rock the lysates gently at 2-8 °C for 30 minutes. Microcentrifuge at 14,000 x g for 5 minutes, and transfer the supernate into a clean test tube. Quantitation of sample protein concentrations using a total protein assay is recommended. Use the lysates immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Apoptosis Array - Immediately before use, remove each membrane to be used from between the protective sheets with a flat-tipped tweezers. **Handle the membranes with gloved hands and flat-tipped tweezers only.**

Detection Antibody Cocktail - Before use, reconstitute the Mouse Apoptosis Detection Antibody Cocktail in 200 µL of deionized or distilled water.

1X Array Buffer 1/8 - Dilute 1 mL of Array Buffer 8 into 12 mL of Array Buffer 1. **Prepare fresh for eash use.**

1X Wash Buffer - If crystals have formed in the concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water.

Chemi Reagent Mix - Chemi Reagents 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 0.5 mL of the resultant mixture is required per membrane.**

1X Streptavidin-HRP - Immediately before use, dilute the Streptavidin-HRP in Array Buffer 1/8. See vial label for dilution factor.

ARRAY PROCEDURE

Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.

1. Prepare all reagents and samples as directed in the previous sections.
2. Pipet 1.0 mL of Array Buffer 1 into each well of the 8-Well Multi-dish. Array Buffer 1 serves as a block buffer.
3. Place each membrane in a separate well. The number on the membrane should be facing upward.

   Note: *Upon contact with Array Buffer 1, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*
4. Incubate for one hour on a rocking platform shaker. Orient the 8-Well Multi-dish so that each membrane rocks end to end in its well.
5. While the arrays are blocking, prepare samples by adding up to 170 µL of each sample to 0.83 mL of Array Buffer 1 in separate tubes. Adjust to a final volume of 1.0 mL with Lysis Buffer 17 as necessary.
6. Aspirate Array Buffer 1 from the wells of the 8-Well Multi-dish and add the prepared samples. Place the lid on the 8-Well Multi-dish.
7. Incubate overnight at 2-8 °C on a rocking platform shaker.

   Note: *A shorter incubation time may be used if optimal sensitivity is not required.*
8. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 8-Well Multi-dish with deionized or distilled water and dry thoroughly.
9. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.
10. For each array, add 20 µL of Detection Antibody Cocktail to 1.0 mL of Array Buffer 1/8. Pipette 1.0 mL per well of diluted Detection Antibody Cocktail into the 8-Well Multi-dish.
11. Carefully remove each array from its wash container. Allow excess 1X Wash Buffer to drain from the array. Return the array to the 8-Well Multi-dish containing the diluted Detection Antibody Cocktail, and cover with the lid.
12. Incubate for 1 hour at room temperature on a rocking platform shaker.
13. Wash each array as described in steps 8 and 9.
14. Pipette 1.0 mL of 1X Streptavidin-HRP into each well of the 8-Well Multi-dish.

ARRAY PROCEDURE *CONTINUED*

15. Carefully remove each membrane from its wash container. Allow excess 1X Wash Buffer to drain from the membrane. Return the membrane to the 8-Well Multi-dish containing the 1X Streptavidin-HRP. Cover the wells with the lid.

16. Incubate for 30 minutes at room temperature on a rocking platform shaker.

17. Wash each array as described in steps 8 and 9.

    Note: *Complete the remaining steps without interruption.*

18. Carefully remove each membrane from its wash container. Allow excess 1X Wash Buffer to drain from the membrane by blotting the lower edge onto paper towels. Place each membrane on the bottom sheet of the plastic sheet protector with the identification number facing up.

19. Pipette 0.5 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

    Note: *Using less than 0.5 mL of Chemi Reagent Mix per membrane may result in incomplete membrane coverage.*

20. Carefully cover with the top sheet of the plastic sheet protector. Gently smooth out any air bubbles and ensure Chemi Reagent Mix is spread evenly to all corners of each membrane. Incubate for 1 minute.

21. Position paper towels on the top and sides of the plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.

22. Leaving membranes on the bottom plastic sheet protector, cover the membranes with plastic wrap taking care to gently smooth out any air bubbles. Wrap the excess plastic wrap around the back of the sheet protector so that the membranes and sheet protector are completely wrapped.

23. Place the membranes with the identification numbers facing up in an autoradiography film cassette.

    Note: *Use an autoradiography cassette that is not used with radioactive isotope detection.*

24. Expose membranes to X-ray film for 1-10 minutes. Multiple exposure times are recommended.

DATA ANALYSIS

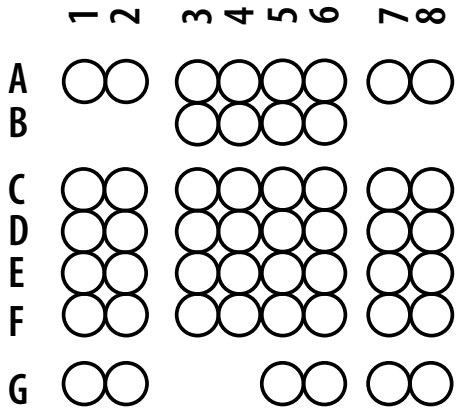
The positive signals seen on developed film can be quickly identified by placing the transparency overlay template on the array image and aligning it with the pairs of reference spots in three corners of each array. The stamped identification number on the array should be placed on the left hand side. The location of controls and capture antibodies is listed in the Appendix.

Note: *Reference spots are included to align the transparency overlay template and to demonstrate that the array has been incubated with Streptavidin-HRP during the assay procedure.*

Pixel densities on developed X-ray film can be collected and analyzed using a transmission-mode scanner and image analysis software.

1. Create a template to analyze pixel density in each spot of the array.
2. Export signal values to a spreadsheet file for manipulation in a program such as Microsoft Excel.
3. Determine the average signal (pixel density) of the pair of duplicate spots representing each analyte.
4. Subtract an averaged background signal from each spot. Use a signal from a clear area of the array or negative control spots as a background value.
5. Compare corresponding signals on different arrays to determine the relative change in analyte levels between samples.

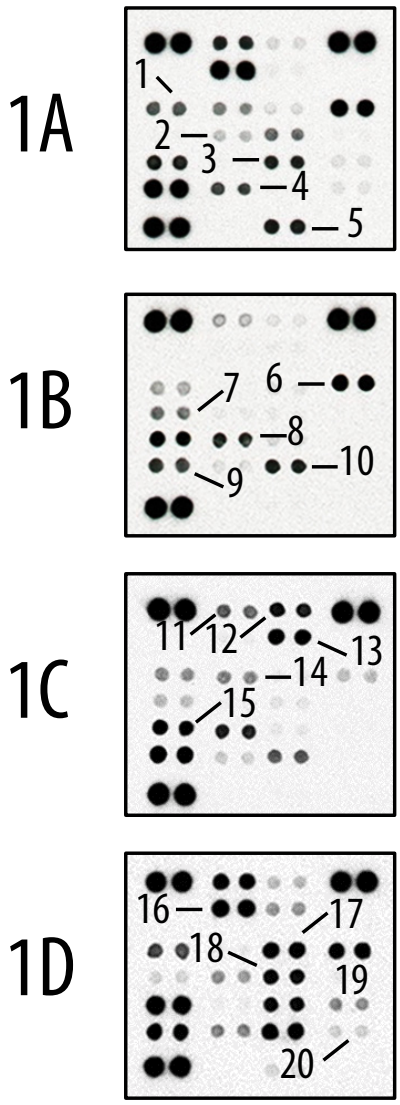
Mouse Apoptosis Array Coordinates



This image is not to scale. It is for coordinate reference only.
Please use the transparency overlay for analyte identification.

PROFILING PROTEINS IN CELL LYSATES

The Mouse Apoptosis Array detects multiple apoptosis-related proteins in cell lysates. Arrays were incubated with cell lysates in amounts indicated below. Data shown are from a 10 minute exposure to X-ray film.



		MEAN PIXEL DENSITY			
		1A	1B	1C	1D
1	Catalase	16,411	7344	10,058	20,125
2	HO-1/HMOX1/HSP32	4282	667	612	8530
3	MCL-1	20,912	1960	1130	25,558
4	SMAC/Diablo	15,607	1649	3041	10,516
5	XIAP	25,039	12	0	2735
6	Fas/TNFRSF6/CD95	36,285	32,718	4841	32,764
7	HIF-1α	509	12,296	5022	3084
8	HSP70/HSPA1A	548	20,252	21,597	822
9	p53	43,413	20,565	35,425	32,234
10	TNF RI/TNFRSF1A	597	23,327	15,984	34,028
11	Bad	22,572	6431	14,499	32,838
12	Bcl-2	2356	1045	24,550	5054
13	Cleaved Caspase-3	1009	513	31,894	9085
14	Claspin	10,142	15	10173	1045
15	HSP60	24,553	27,513	28,239	44,589
16	Bcl-x	44,499	239	487	38,736
17	Cytochrome c	1747	1347	741	33,406
18	HO-2/HMOX2	11,843	541	1421	26,855
19	p27/Kip1	1955	27	94	9477
20	TRAIL R2/TNFRSF10B	1462	68	12	3176

Figure 1A: A20 mouse rectal carcinoma cell lysate (650 µg).

Figure 1B: SW/3T3 Swiss albino mouse embryo fibroblast cell lysate (300 µg).

Figure 1C: Ba/F3 mouse lymphocyte cell lysate (400 µg).

Figure 1D: Hepa 1-6 mouse liver epithelial cell lysate (650 µg).

PROFILING PROTEINS IN UNTREATED AND TREATED SAMPLES

The Mouse Apoptosis Array detects multiple apoptosis-related proteins in untreated and treated cell lysates. Arrays were incubated with 500 µg cell lysate. Data shown are from a 10 minute exposure to X-ray film. Profiles of mean spot pixel density were created using a transmission-mode scanner and image analysis software.

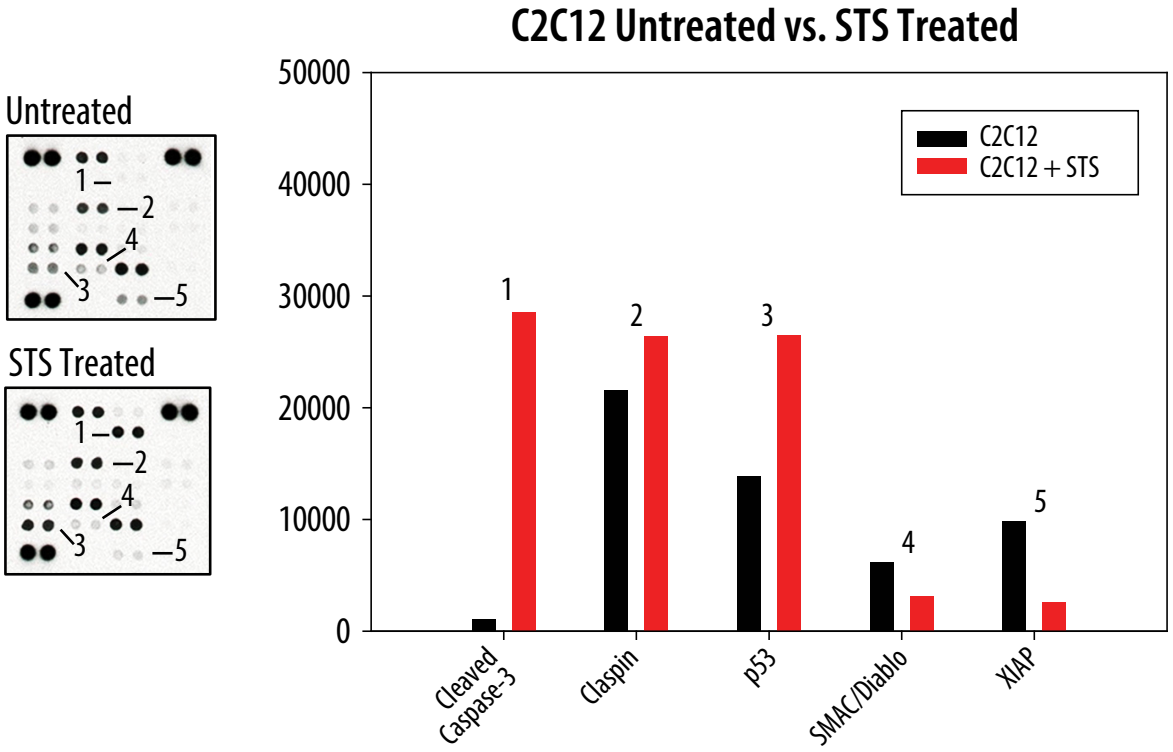


Figure 2: Lysates from C2C12 mouse muscle myoblast cells were left untreated or treated with 1 µM STS for 3 hours.

APPENDIX

Refer to the table below for the Mouse Apoptosis Array coordinates.

Coordinate	Analyte	Entrez Gene ID	Alternate Nomenclature
A1,2	Reference Spots	N/A	————
A3,4	Bad	12015	BBC6, BCL2L8
A5,6	Bcl-2	12043	Bcl2
A7,8	Reference Spots	N/A	————
B3,4	Bcl-x	12048	BCL2L1
B5,6	Caspase-3, cleaved	12367	CASP3, CPP32
C1,2	Catalase	12359	Cas1, CAT, Cs-1
C3,4	Claspin	269582	CLSPN
C5,6	Cytochrome c	13063	CYCS, cyt c
C7,8	Fas/TNFRSF6/CD95	14102	APT1, FAS1
D1,2	HIF-1α	15251	HIF1A
D3,4	HO-1/HMOX1/HSP32	24451	————
D5,6	HO-2/HMOX2	15369	————
D7,8	HSP27	15510	————
E1,2	HSP60	110907	HSP65, HSPD1
E3,4	HSP70/HSPA1A	15511	HSP72
E5,6	MCL-1	17210	BCL2L3
E7,8	p27/Kip1	12576	CDKN1B
F1,2	p53	22059	BCC7, LFS1, TP53, TRP53
F3,4	SMAC/Diablo	66593	————
F5,6	TNF RI/TNFRSF1A	21937	CD120a
F7,8	TRAIL R2/TNFRSF10B	21933	DR5
G1,2	Reference Spots	N/A	————
G5,6	XIAP	11798	BIRC4, IAP3
G7,8	Negative Control	N/A	————