ab193661 – Human HSP Antibody Array - Membrane (9 Targets)

Instructions for Use

For the simultaneous detection of the relative levels of 9 Heat shock protein markers in cell lysates

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 14 December 2016

Table of Contents

1. BACKGROUND

Abcam's Human HSP Antibody Array - Membrane (9 Targets) ab193661 can be used for the simultaneous detection of the relative levels of 9 Heat shock protein markers in cell lysates.

Targets: HSP27, HSP32, HSP40, HSP60, HSP70, HSP90, GRP75, Ubiquitin+1, HSP10

Heat shock proteins (HSP) are a family of functionally related molecular chaperones, which play critical roles in protein folding, intracellular trafficking of proteins, and coping with proteins denatured by heat shock and other stresses. HSPs are found in virtually all living organisms, from bacteria to humans.

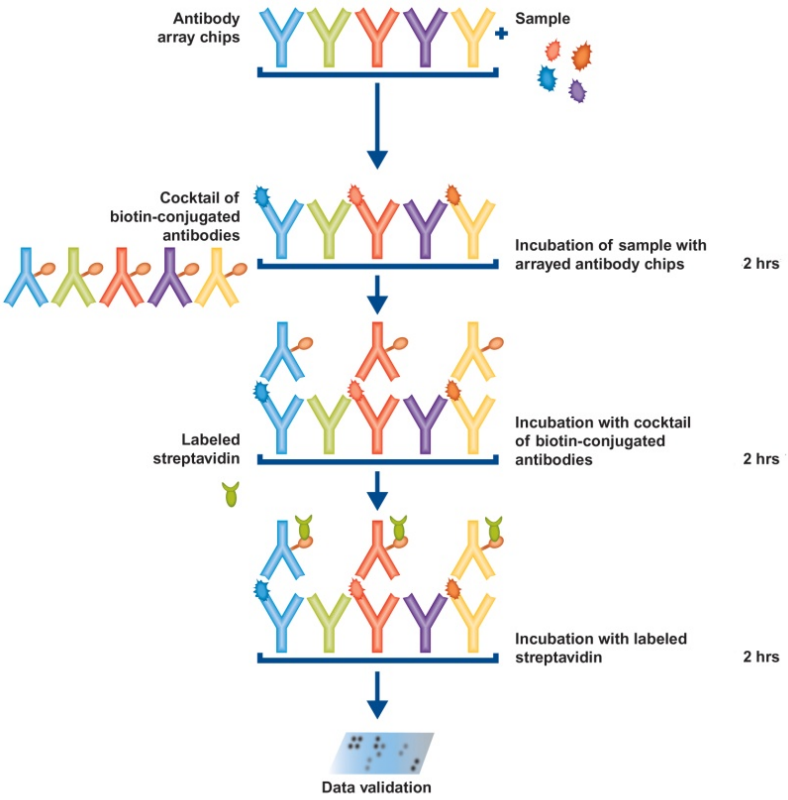
HSP family includes 5 major classes according to their molecular weights, i.e., the small heat-shock proteins (sHSPs), HSP33, HSP60, HSP70 and HSP90/ HSP100. The smaller 8-kD protein ubiquitin, which marks proteins for degradation and is regarded as co-chaperone, also belongs to HSP family.

In addition to their roles in protein trafficking and and stress response, HSPs are also important in cardiovascular functions and modulation of immune responses. Some HSPs have been under investigation as therapeutic targets in cancer.

With Abcam's Human HSP Array – Membrane ab193661, researchers can now simultaneously detect the relative level of 9 HSP related proteins in cell lysates. By monitoring the changes in protein levels in different experimental model systems, researchers can study pathway activation without spending excess time and effort in performing immunoprecipitations and/or Western Blotting.

Each array membrane is pre-printed with capture antibodies; treated or untreated cell lysate is then added to each membrane. After extensive washing, the membranes are incubated with a cocktail of biotin-conjugated anti-apoptotic protein antibodies. After incubation with HRP-Streptavidin, the signals are visualized by chemiluminescence.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC immediately upon receipt.

Once thawed, for short-term storage, store array membranes, 1X Blocking Buffer and Protease Inhibitor Cocktail at ≤ -20°C, and all other component at 2-8°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. MATERIALS SUPPLIED

Item	Quantity			Storage Condition (Before Preparation)
	2X Membranes	4X Membranes	8X Membranes	
Human HSP Antibody Array Membranes (C1)	2X C1 Membranes	4X C1 Membranes	8X C1 Membranes	-20ºC
Biotinylated Antibody Cocktail (C1)	1X C1 Vial	2X C1 Vials	4X C1 Vials	-20ºC
1000X HRP - Conjugated Streptavidin	1X 50 µL	1X 50 µL	1X 50 µL	-20ºC
1X Blocking Buffer	1X 25 mL	1X 25 mL	2X 25 mL	-20ºC
20X Wash Buffer I	1X 10 mL	1X 10 mL	1X 20 mL	-20ºC
20X Wash Buffer II	1X 10 mL	1X 10 mL	1X 20 mL	-20ºC
2X Cell Lysis Buffer Concentrate	1X 5 mL	1X 5 mL	1X 5 mL	-20ºC
Detection Buffer C	1X 1.5 mL	1X 1.5 mL	1X 2.5 mL	-20ºC
Detection Buffer D	1X 1.5 mL	1X 1.5 mL	1X 2.5 mL	-20ºC
Protease Inhibitor Cocktail	1X 60 µL	1X 60 µL	2X 60 µL	-20ºC
8-Well Incubation Tray with Lid	1 Unit	1 Unit	1 Unit	-20ºC

The kit also includes plastic sheets, a booklet, an array template and a packing list.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Pipettors, pipet tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Tissue Paper, Blotting Paper or Chromatography Paper
- Adhesive Tape or Plastic Wrap
- Distilled or De-ionized Water
- Chemiluminescent blot documentation system (CCD Camera, X-Ray Film and a suitable film processor, Gel documentation system, or other chemiluminescent detection system capable of imaging a Western blot)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

Handling Array Membranes

- Always use forceps to handle membranes, and grip the membranes by the edges only. Flat-tipped forceps are best for handling membranes.
- Never allow array membranes to dry during experiments.
- Avoid touching array membrane by hand, tips or any sharp tools.

Incubation and Washes

- Completely cover membranes with sample or buffer during incubation, and cover eight-well tray with lid to avoid drying.
- Avoid bubbles and foaming during incubation steps.
- Perform all incubation and wash steps under gentle shaking (1-2 cycles/second).
- The following incubations may be done overnight at 2-8°C: all wash steps, Step 12.3. (sample incubation), Step 12.7. (biotin-Ab incubation) and Step 12.10. (HRP-Conjugated Streptavidin incubation).

9. REAGENT PREPARATION

Thaw all reagents to room temperature immediately before use.
Reagents should only be used in their 1X working concentration.

9.1. **1X Cell Lysis Buffer**

2X Cell lysis buffer should be diluted 2-fold with deionized or distilled water before use.

NOTE: Proceed to 9.2. before completing the rest of the 2X Cell Lysis Buffer preparation.

Add 20 µL of prepared 100X Protease Inhibitor Cocktail Concentrate (bring the tube to room temperature to thaw the solution before use) into 1.98 mL 1X Lysis Buffer before use. Mix well.

9.2. **Protease Inhibitor Cocktail**

Briefly spin down the Protease Inhibitor Cocktail tube before use. Add 60 µL of 1X Lysis Buffer into the vial to prepare a 100X Protease Inhibitor Cocktail Concentrate.

NOTE: Be sure to complete 2X Cell Lysis Buffer preparation before proceeding to 9.3.

9.3. **1X Wash Buffer I**

If the Wash Buffer Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into 380 mL of deionized or distilled water to yield 400 mL of 1X Wash Buffer I.

9.4. **1X Wash Buffer II**

If the Wash Buffer Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into 380 mL of deionized or distilled water to yield 400 mL of 1X Wash Buffer II.

9.5. **1X Biotinylated Antibody Cocktail**

Briefly centrifuge each vial before use. Add 150 µL of 1X Blocking Buffer to the tube. Mix gently and transfer the entire mixture to a tube containing 1.80 mL of 1X Blocking Buffer to prepare 1X Cocktail of Biotin-Conjugated Antibody Mix.

9.6. **1X HRP-Conjugated Streptavidin**

Briefly spin down the HRP-Conjugated Streptavidin to remove any liquid from the vial cap and pipette up and down to mix gently before use. Prepare 1X HRP Conjugated Streptavidin. Example: add 5 µL of HRP-Conjugated Streptavidin concentrate into a tube with 4.995 mL 1X Blocking Buffer and mix gently. Use immediately, do not store the 1X Streptavidin for next day use.

NOTE: mix tube containing 1,000X HRP-Conjugated Streptavidin well before use since precipitation may form during storage.

- Blocking buffer and Detection Buffers C and D are supplied at working concentrations.

10. SAMPLE PREPARATION AND STORAGE

10.1 Remove supernatant from cell culture for attached cells, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1500 rpm for 10 minutes) making sure to remove any remaining PBS before adding Lysis Buffer. Solubilize the cells at $2x10^7$ cells/mL in 1X Lysis Buffer containing Protease Inhibitor Cocktail (prepared in step 9.1. of Reagent Preparation). Pipette up and down to resuspend cells and rock the lysates gently at 2–8°C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.

10.2 It is recommended that sample protein concentrations be determined using a total protein assay such as the BCA Assay. Before applying to the membranes, all lysates should be diluted at least 5-fold with 1X Blocking Buffer. For Abcam's Human HSP Array – Membrane ab193661, 200 µg/mL to 600 µg/mL of total protein from cell lysates (after dilution with 1X Blocking Buffer) should be used for incubation. Lysates should be used immediately or aliquoted and stored at -80°C. Thawed lysates should be kept on ice prior to use.

NOTE: If you experience *high background, you may further dilute your samples.*

11. ARRAY MAP

POS – Positive Control
NEG – Negative Control
UBIQ – Ubiquitin
HSP – Heat Shock Proteins

Array Map for Human HSP Antibody Array – Membrane C1 (9 Targets) ab193661

	A	B	C	D	E	F	G
1	POS	NEG	HSP27	HSP32	HSP40	HSP60	HSP70
2	HSP90	GRP75	UBIQ + 1	HSP10	NEG	NEG	POS

12. ASSAY PROCEDURE

Please prepare all reagents immediately prior to use. All incubations and washes must be performed under gentle rotation/rocking.

12.1. Place each membrane printed side up into the 8-well tray provided in the kit (top left corner marked with "-").

NOTE: The printed side should be facing upward.

12.2. Add 1 mL 1X Blocking Buffer and incubate at room temperature with gentle shaking for 30 minutes to block membranes.

12.3. Decant 1X Blocking Buffer from each well. Add 1 mL of diluted sample onto each array membrane, and cover with the lid. Incubate at 2–8°C overnight on a rocker or shaker (low speed 1-2 cycles/sec). Dilute sample using 1X Blocking Buffer.

NOTE: 1) Dilute cell lysates at least 5-fold with 1X Blocking Buffer to avoid high background.

NOTE: 2) Optimal sample dilution factors should be determined empirically. More sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.

NOTE: 3) Incubation may be done at room temperature for 4 hours, but this may cause lower signals.

12.4. Decant the samples from each well, and wash 3 times with 1.5 mL of 1X Wash Buffer I at room temperature with shaking, 5 minutes per wash.

12.5. Wash 2 times with 1.5 mL of 1X Wash Buffer II at room temperature with shaking, 5 minutes per wash.

12.6. Carefully remove wash buffer from each well containing array membranes.

12.7. Add 1 mL of diluted Cocktail of Biotin-Conjugated Antibody Mix to each membrane. Incubate at 2–8°C with gentle shaking overnight.

NOTE: Incubation may be done at room temperature for 2 hours.

12.8. Wash membranes as directed in Steps 12.4., 12.5. and 12.6.

12.9. Add 1.5 mL of 1X HRP-Conjugated Streptavidin to each membrane.

12.10. Incubate HRP-Conjugated Streptavidin at room temperature for 1.5 hours.

NOTE: Incubation may be done overnight at 2-8°C.

12.11. Wash as directed in Steps 12.5. and 12.6.

12.12. Add 250 µL of Detection Buffer C and 250 µL of Detection Buffer D for one membrane; mix both solutions; Drain off excess wash buffer by holding the membrane vertically with forceps. Place membrane protein side up ("-" mark is on the protein side top left corner) on a clean plastic sheet (provided in the kit). Pipette the mixed Detection Buffer on to the membrane and incubate at room temperature with gentle shaking for 2 minutes. Ensure that the detection mixture is completely and evenly covering the membrane without any air bubbles.

12.13. Drain off excess detection reagent by holding the membrane vertically with forceps and touching the edge against a tissue. Gently place the membrane, protein side up, on a piece of plastic sheet ("-" mark is on the protein side top left corner). Cover the array with another piece of plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane.

12.14. Detect signal directly from membrane using chemiluminescence imaging system or expose to x-ray film to detect signal using film developer. Expose the membranes for 40 Seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce exposure time (e.g. 5–30 seconds). If the signals are too weak, increase

exposure time (e.g. 2–20 minutes or overnight). Or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

12.15. Save membranes at ≤ –20 °C for future reference.

13. CALCULATIONS

Membrane signals are normally detected by a chemiluminescence imaging device. Membranes also can be exposed to x-ray film at room temperature. A biotinylated protein provides positive signals, which can be used to identify the orientation and to normalize the results from different wells being compared.

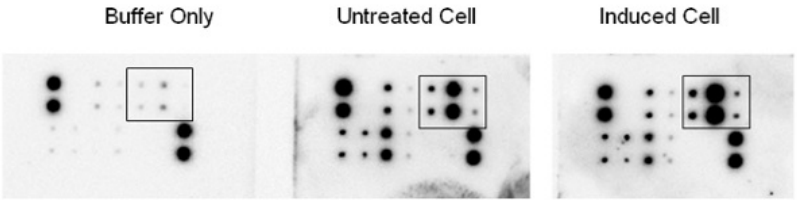
By comparing the signal intensities, relative expression levels of target proteins can be made. The intensities of signals can be quantified by densitometry. The positive controls can be used to normalize the results from different membranes being compared.

Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies.

One important parameter is the baseline signal response. To obtain the best results, we suggest that several exposures be attempted. We also strongly recommend using a negative control in which the sample is replaced with an appropriate mock buffer according to the array protocol, particularly during your first experiment.

14. TYPICAL DATA

Typical results obtained with Abcam Antibody Arrays:



Heat shock protein profiling in induced HepG2 cultured cells.
HepG2 cells were treated with 100µM hydrogen peroxide for 24 hours 500 µg of cell lysates from both untreated and induced HepG2 cells were incubated overnight with Abcam Human HSP Array – Membrane ab196661. Control membrane was incubated with blocking buffer. The membranes were then washed and cocktail of biotinylated antibodies was used to detect bound proteins. After incubation with HRP-Conjugated Streptavidin, the signals were visualized by chemiluminescence.

15. TROUBLESHOOTING

Problem	Cause	Recommendation
Weak or no signal	Taking too much time for detection	Entire detection process must be completed in 30 minutes
	Film developer does not work properly	Fix film developer
	Did not mix HRP-Conjugated Streptavidin well before use	Mix tube containing HRP-Conjugated Streptavidin well before use since precipitates may form during storage.
	Sample is too dilute	Increase sample concentration
	Other	Reduce blocking concentration by diluting in 1X Wash Buffer II
		Slightly increase HRP concentration
		Slightly increase biotin-antibody concentration
		Expose film overnight
Uneven signal	Bubbles formed during incubation	Remove bubbles during incubation
	Membranes were not completely covered by solution	Completely cover membranes with solution
High background	Exposure to x-ray film is too long	Decrease exposure time.
	Membranes were allowed to dry out during experiment	Completely cover membranes with solution during experiment
	Sample is too concentrated	Increase sample dilution

16. NOTES

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