

Whatman®

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FAST® Macro Protocol Human I / Mouse I

TABLE OF CONTENTS

I. Product Description	3
II. Kit Components and Storage Conditions	3
A. Components	3
B. Storage	3
III. Additional Materials and Equipment Needed	4
IV. Important Considerations	4
A. Sensitivity	4
B. Sample Volume	5
V. Protocol	6
A. Processing the macroarray	6
B. Interpretation of Results	9
FAST® Macro Human I Array Map	9
FAST Macro Mouse I Array Map	10
Appendix	10
Ordering Information	Back Cover

I. PRODUCT DESCRIPTION

Whatman FAST® Macro membrane-based antibody arrays are designed as a tool to simultaneously evaluate the relative abundance of 20 different cytokines between different biological samples, such as a disease state versus a normal sample, using chemiluminescent detection. FAST Macro kits contain anti-cytokine antibodies arrayed on nitrocellulose, a surface which is well known for its high protein binding capacity.

Each anti-cytokine antibody is arrayed in triplicate. In addition to having one corner of the membrane notched, each membrane includes positive chemiluminescent control spots for array orientation purposes. The kit also includes biotinylated detection antibodies, blocking and washing buffers. Assay processing time is approximately 5 hours.

II. KIT COMPONENTS AND STORAGE CONDITIONS

A. Components

- 4 or 8, antibody-arrayed 19 x 19 mm nitrocellulose membranes
- FAST Macro Blocking Buffer
- 10X FAST Macro Wash Buffer
- 10X biotinylated antibodies cocktail in 0.5mg/ml BSA, 1X tris-buffered saline (TBS)
- Plastic processing tray
- Protocol

B. Storage

- Membranes: store membranes in closed foil pouch at room temperature and room relative humidity.
- FAST Macro Blocking Buffer: store at -20°C.
- Biotinylated antibodies cocktail: store at -20°C.
- All other components: store at room temperature.

III. ADDITIONAL MATERIALS AND EQUIPMENT NEEDED (not provided in FAST® Macro Kit)

- Forceps
- Micro-pipettors and tips
- Orbital or back-and-forth shaker
- 1X phosphate-buffered saline pH 7.4
- Streptavidin-horseradish peroxidase (Amersham Biosciences, catalog # RPN1231)
- ECL™ detection reagents (Amersham Biosciences, catalog # RPN2109)

NOTE: The use of alkaline phosphatase chemiluminescent detection materials involving dioxetane substrates is not recommended.

- Plastic wrap
- X-ray film cassette
- Autoradiography film (Kodak BioMax MR film, catalog #870 1302)
- Film processor

OPTIONAL:

- Chip Clip™ slide holder (S&S catalog #10 486 081)
- Dual-well incubation chambers (S&S catalog #10 486 087)
- Standard, untreated glass microscope slide(s)

IV. IMPORTANT CONSIDERATIONS

A. Sensitivity

Sensitivity levels are dependent on antigen incubation time and film exposure times. The stated cytokine levels are obtained with 2 hour antigen incubation and one hour exposure times.

Sensitivity Levels for FAST Macro Human I and Mouse I Menus:

Sensitivity levels were based on a visual comparison of autoradiography films of antigen-incubated membranes to negative control membranes (no antigen added) from four different experiments – three using 1X PBS, 1mg/ml BSA, 0.05% Tween 20 as the diluent and one with 1X PBS, 10% FCS as the antigen diluent.

*Sensitivity Levels for
FAST Macro Human I Menu*

Specificity	Sensitivity (pg/ml)	Specificity	Sensitivity (pg/ml)
Eotaxin	200	IL10	250
GM-CSF	20	IL13	2000
IFN γ	2000	IP-10	150
IL1 α	20	MCP-1	20
IL1 β	20	MCP-2	20
IL2	100	MCP-3	100
IL4	150	MCP-4	150
IL5	150	MIP-1 α	150
IL6	20	Rantes	40
IL8	15	TNF α	20

*Sensitivity Levels for
FAST Macro Mouse I Menu*

Specificity	Sensitivity (pg/ml)	Specificity	Sensitivity (pg/ml)
MCSF	62.5	IL10	65.5
GM-CSF	31.2	IL13	250
IFN γ	250	IL12p70	250
IL3	31.2	MCP-1	31.2
IL1 β	125	MCP-5	62.5
IL2	125	MIP-2	62.5
IL4	125	VEGF	62.5
IL5	62.5	MIP-1 α	250
IL6	62.5	Rantes	31.5
IL12p40	62.5	TNF α	62.5

B. Sample Volume

The sample volume required when incubating in the plastic processing tray is 650 μ l. Alternatively, the Whatman Chip Clip™, used in conjunction with a dual well incubation chamber and a standard untreated glass microscope slide, can serve as an incubation tray if the sample volume is limited (400 – 500 μ l). All washing and labeling steps for such a sample would still have to be carried out in the plastic processing tray.

V. PROTOCOL

NOTE: The location of the arrayed anti-cytokine antibodies on the nitrocellulose membrane is initially visible due to the presence of a dye. This allows the researcher to note the array position with respect to the notched corner of the membrane. The dye does not interfere with the assay and is removed from the membrane when the membrane is wetted out in 1X PBS.

NOTE: Never handle the membranes without wearing gloves or using a clean forceps.

NOTE: Use Universal Safety Precautions whenever handling any human body fluids.

A. Processing the macroarray

1. Orient the membrane so that the notched corner of the printed side is at the lower right. Using a pencil, label the membrane below the last row of spots and near the notched corner.
2. Gently pick up a 19 x 19 mm membrane, printed side up, at the notched end with clean forceps and transfer to a compartment in the 2 x 4 plastic tray to which 2.5 ml 1X PBS has been added. Place one membrane per compartment.
3. Once the membrane is wet and the dye is removed (approximately 2-3 minutes), using a forceps, pick up the membrane at the notched corner and turn over so that arrayed side is facing downwards. Leave membrane facing downward throughout the remainder of the blocking, incubation, and wash steps. Remove the PBS with a pipette. Block the membrane by adding 650 μ l of FAST Macro Blocking buffer per membrane. Close the tray's lid and agitate the slide for 30 minutes on an orbital shaker or a back-and-forth rocker.
4. Remove the blocking solution with a pipette and rinse the membrane with 650 μ l of 1X PBS/0.05% Tween 20. Remove the rinse solution with a pipette.
5. Add 650 μ l of sample/antigen solution per membrane. Close the lid and incubate for 2 hours with agitation.

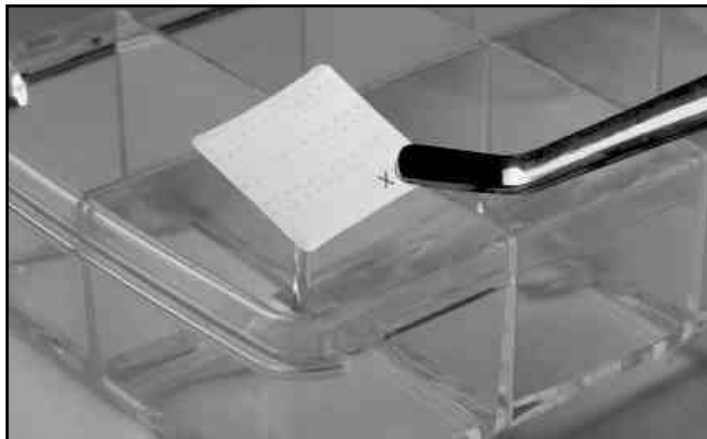
6. To eliminate cross contamination, remove the antigen solution by pipetting; do not decant the antigen solution.

NOTE: Never handle the membranes without wearing gloves or using clean forceps.

NOTE: For sample volumes of 400 - 500 μ l, incubate membrane using a standard glass microscope slide and the Chip Clip™ slide holder. See Appendix for instructions.

7. Wash each membrane by adding 2 ml of 1X FAST Macro Wash Buffer. Agitate for 5 minutes. Repeat wash steps for a total of 3 times. During the wash steps, prepare the biotinylated antibody cocktail.

Figure 1. FAST®Macro array is placed in processing tray for wet-out



8. Remove the biotinylated antibody cocktail from the freezer and thaw at room temperature. Dilute an appropriate amount of the biotinylated cocktail 1:10 with 1X FAST Macro Wash Buffer. Mix well.
9. Add 650 μ l of 1X biotinylated antibody cocktail diluted in FAST Macro Wash Buffer to each membrane. Close the lid and agitate for one hour.
10. Carefully decant biotinylated antibody cocktail solution and wash the membrane with 2 ml of FAST Macro Wash Buffer. Agitate for 5 minutes. Repeat wash steps for a total of 3 times.
11. Remove wash buffer and add 2 ml of a 1:5000 dilution of streptavidin-horseradish peroxidase (SA-HRP) diluted in FAST Macro Wash Buffer to each tray compartment containing a membrane. Agitate for 45 minutes.
12. Remove the SA-HRP and wash the membrane in 2 ml wash buffer for a total of 4 washes. Each wash is for 5 minutes with agitation.
13. Drain excess wash buffer from membrane and place membrane, protein side up, on plastic wrap. Add 0.250 ml of ECL detection reagent (mix an equal volume of reagent 1 to reagent 2) to membrane and incubate for 1 to 2 minutes.
14. Drain off excess liquid and transfer membrane to a clean piece of plastic wrap. Using the plastic wrap, seal the membranes making sure to remove all air bubbles.
15. Place wrapped membranes in a film cassette, add a piece of Kodak BioMax autoradiography film and expose for 15 minutes.
16. Develop film and adjust exposure time depending on signal intensity.

NOTE: Alternatively, imaging may be carried out using a phosphor imager such as the Typhoon™ Imager from Amersham Biosciences.

B. Interpretation of Results

The membrane is oriented by the notch in the lower right corner and the position of the positive control spots. To determine which growth factor/cytokine is represented by a positive signal, consult the array map for the kit you are using.

Figure 2. FAST® Macro Human I Array Map.

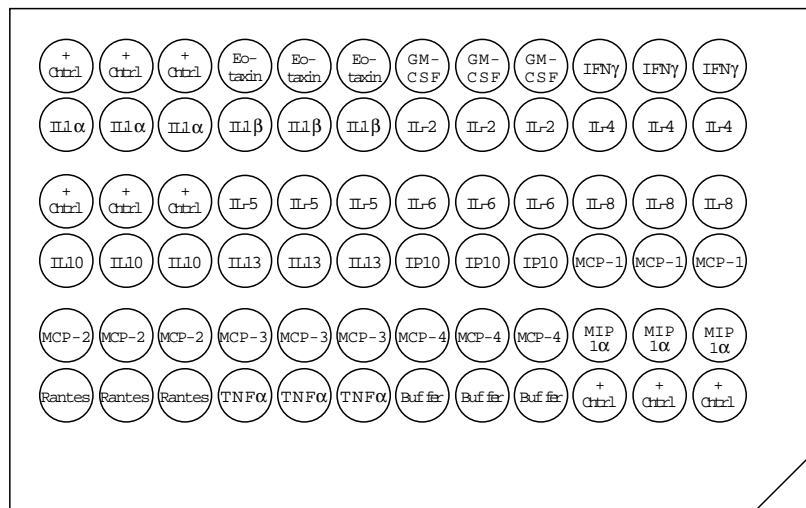
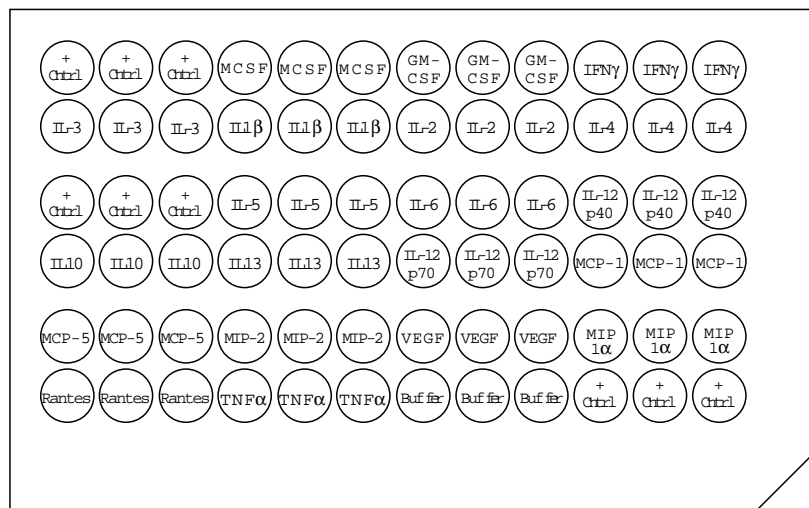


Figure 3. FAST®
Macro Mouse I
Array Map.



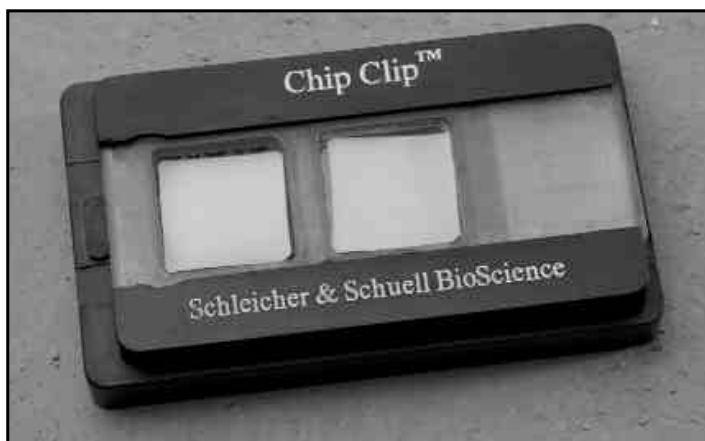
APPENDIX

Incubation of 400 - 500 μ l samples using the Chip Clip™ Slide Holder

1. Place a Whatman dual-well incubation chamber on top of a clean, untreated microscope slide.
2. Insert the slide with chamber into the Chip Clip.
3. Gently remove membrane from plastic processing tray at the notched corner using a forceps, and place, printed side facing downwards, into one of the wells of the incubation chamber (see figure 4).

4. Add 400 – 500 μ l of sample/antigen solution per membrane.
5. Place a wet paper towel in a resealable bag. Cover the Chip Clip with a 96-well plate lid and slide the Chip Clip assembly into the resealable bag and seal the bag.
6. Incubate for 2 hours with agitation.
7. Remove the antigen solution by pipetting.
8. Return membrane to plastic processing tray, printed side down, holding it with a forceps at the notched corner.
9. Proceed with washing and labeling steps as described in step 8 of the main protocol.

Figure 4. Incubation of two FAST[®] Macroarrays in ChipClip[™] Slide Holder.



Ordering Information

Description	Qty/Pkg	Item #
FAST[®] Macro Human I Kit (4 arrays)	1	10 486 151
FAST Macro Human I Kit (8 arrays)	1	10 486 152
FAST Macro Mouse I Kit (4 arrays)	1	10 486 166
FAST Macro Mouse I Kit (8 arrays)	1	10 486 167
Chip Clip Slide Holder	1	10 486 081
Dual Well Incubation Chambers	10	10 486 087

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