PEPperPRINT
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Quick Start Guide

PEPperCHIP® Immunoassay Protocol

Day 1

Assemble
incubation tray

Hydrate
wash buffer
15 min

Block
blocking buffer
30 min

Incubate
secondary antibody
45 min

Wash
wash buffer
3 x 1 min or 2 x 10 sec

Disassemble
incubation tray

Dip
dipping buffer
2-3 times

Air dry
from top to bottom

Scan
microarray

Assemble
incubation tray

Equilibriate
staining buffer
15 min

Incubate
your primary sample
overnight, 4°C

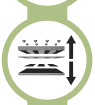
Day 2

Wash
wash buffer
3 x 1 min or 2 x 10 sec

Incubate
secondary antibody
45 min

Wash
wash buffer
3 x 1 min or 2 x 10 sec

Disassemble
incubation tray



Dip
dipping buffer

Air dry
from top to bottom

Scan
microarray

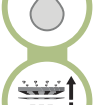
Assemble
incubation tray

Equilibriate
staining buffer
15 min

Incubate
control antibody
45 min

Wash
wash buffer
3 x 1 min or 2 x 10 sec

Disassemble
incubation tray



Dip
dipping buffer
2-3 times

Air dry
from top to bottom

Scan
microarray

Approximate experimental time for each day: 2.5-3 hours

Watch our video tutorial:

youtu.be/-kc5MV8z8GY

Protocols

Dowload and read

pepperprint.com/technology/downloads-and-protocols

Watch

- Animated tutorial: youtu.be/-kc5MV8z8GY
- JoVE video: youtu.be/sY6vFbTBWoU

Buffers

Wash buffer

PBS, pH 7.4
+ 0.05% Tween 20 for linear peptides
+ 0.005% Tween 20 for cyclic constrained peptides

Blocking buffer

Rockland Blocking buffer MB-070
or
Wash buffer with 1% BSA

Staining buffer

Wash buffer with 10% blocking buffer

Dipping buffer

1 mM Tris, pH 7.4

General recommendations

- Only touch the microarray slide at the rims with your fingers, or use plastic forceps (in the area of microarray number).
- Place the microarray number appearing mirror-inverted in the upper right corner.
- Use the dark incubation tray lid to prevent dye bleaching.
- Use an orbital shaker for optimal surface wetting (avoid rocking incubation, which might increase background and artefacts).
- Pipette with the tip being placed close to the incubation tray wall; avoid touching the microarray content.

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Reference Tables

PEPperCHIP® Immunoassay Protocol

Sample volumes and dilutions

Sample type or secondary antibody	Range	Recommended starting dilution or concentration	PEPperCHIP® array format (no. of array copies/chip)	Minimum assay volume
Purified antibody	1 - 500 µg/mL	1 µg/mL	1	1,500 µL
Serum (IgG, IgM, IgA)	1:10 - 1:10,000	1:1,000	2	700 µL
Serum (IgE)	1:2 - 1:10,000	1:5	3	400 µL
Cerebrospinal fluid	1:10 - 1-10,000	1:100	4	350 µL
Secondary antibody	1:500 - 1:10,000	1:100	5	200 µL
Anti-HA control antibody	1:2,000	1:2,000	16	100 µL

Troubleshooting

Issue	Possible reason	Solution
High background signal	Insufficient washing	Extend washing steps.
	Non-homogenous sample	Centrifuge sample prior to dilution.
	Use of strongly charged fluorescent dye	Switch to a combination of either Cy3/Cy5 or DL680/DL800.
Weak signal intensities	Low-affinity ineractions or high off-target binding rates	Reduce washing steps or re-incubate the same array copy with a lower dilution (for serum) or a higher concentration (for antibody) of sample.
	Insufficient blocking	Use the recommended Rockland Blocking buffer for optimal signal-to-noise ratio.
Artefacts on the microarray scan	Portions of the microarray were left uncoated with sample or buffer	Ensure that the array is always fully coated with sample or buffer during the immunoassay.
	Array was poorly rinsed and dried	Dip the microarray chip according to the protocol and dry it immediately with a pressurized air stream.
	Incorrect placement of the silicone seal	Place and align the silicone seal precisely onto the microarray chip.
Scratches or blurry areas on the microarray scan	Accidental contact with the printed surface of the microarray chip	Avoid any contact to the microarray surface; only touch the microarray chip at the rim or use plastic forceps for handling.

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