
Protocol for Microwave-assisted Immuno-labeling

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SOLUTIONS:

A: 10 mM Phosphate buffer

0.01 M phosphate:

KH_2PO_4 (monobasic stock, 0.4M) 1.75 ml

K_2HPO_4 (dibasic stock, 0.4M) 4.5 ml

Q.S. with nH_2O pH 7.2 250.0 ml

pH to 7.2

B: 10 mM Phosphate buffer + 50 mM NH_4Cl

0.1 M Phosphate buffer 10.0 ml

NH_4Cl (Sigma A-4514, MW 53.5) 0.27 g

Q.S. to 100 ml with nH_2O

PROTOCOL:

[] Rinse sample in **Buffer A** for 5 min.

[] Wash 2 times at 1 minute at 0% power, 40 seconds at 100% power, 3 minutes at 0% power in **Buffer A**.

[] Wash in **Buffer B** 1 minute at 0% power, 40 seconds at 100% power, 3 minutes at 0% power to quench free aldehydes formed in glutaraldehyde fixative.

[] Wash in PBS one time 1 minute at 0% power, 40 seconds at 100% power, 3 minutes at 0%

power.

IMMUNO-LABELING IN THE MICROWAVE

1. Apply blocking buffer for 2 minutes at 100% power, 2 minutes at 0% power and 2 minutes at 100% power at 37°C set point.
 2. Remove excess buffer from the slide.
 3. Apply appropriate dilution of primary antibody and incubate at 37°C for 2 minutes at 100% power, 2 minutes at 0% power, 2 minutes at 100% power and 2 minutes at 0% power.
 4. Wash with PBS three times 40 seconds at 100% power
 5. Apply secondary antibody and incubate at 37°C for 2 minutes at 100% power, 2 minutes at 0% power, 2 minutes at 100% power and 2 minutes at 0% power.
 6. Wash with PBS three times 40 seconds at 100% power.
 7. Rinse well with distilled water, mount in antifade mounting media and observe.
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