

Microwave Assisted Immunolabeling

Principle:

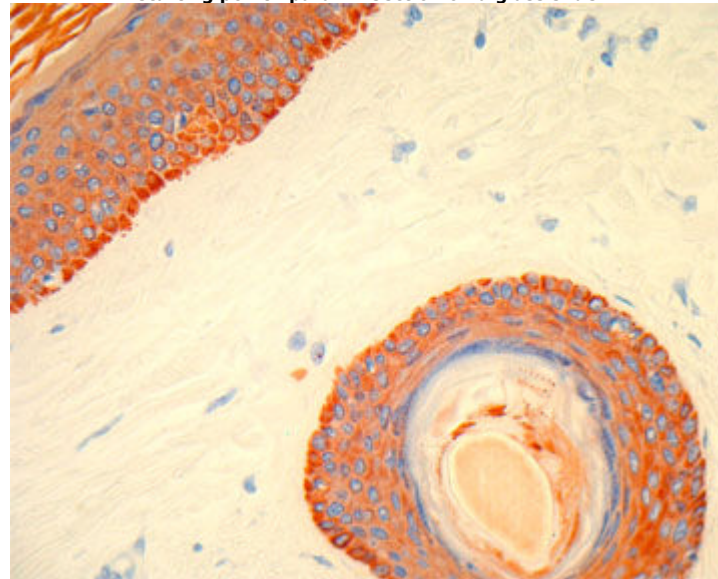
The immunolabeling of slides, coverslips or free-floating sections is a time consuming process. On the bench the whole process will take a few hours or days. Immunolabeling can be direct, indirect or a sandwich assay and can use fluorescent or enzyme based chromogens. Various blocking steps are employed and numerous rinses are incorporated in the overall process. Many steps and different reagents are therefore being used in a typical immunoassay.

Microwave-assisted immunolabeling has demonstrated the ability to greatly shorten turn-around times regardless of the labeling protocol used. This statement has been validated through numerous workshops and recent publications. The ability to use microwave radiation effectively has evolved through better control of the cavity environment during microwave exposure. Slow controlled sample heating, on the order of 2-3°C a minute, are the norm.

How to Begin:

The starting sample can be a free-floating frozen or Vibratome section, paraffin section, tissue culture plate or small embryo.

starting point: paraffin section on a glass slide.



Microwave-assisted immunolabeling of human skin with a universal strepavidin/biotin immunostaining system. A polyclonal cytokeratin wide spectrum primary antibody was used along with AEC as the chromagen. Total staining time <60 minutes in microwave.

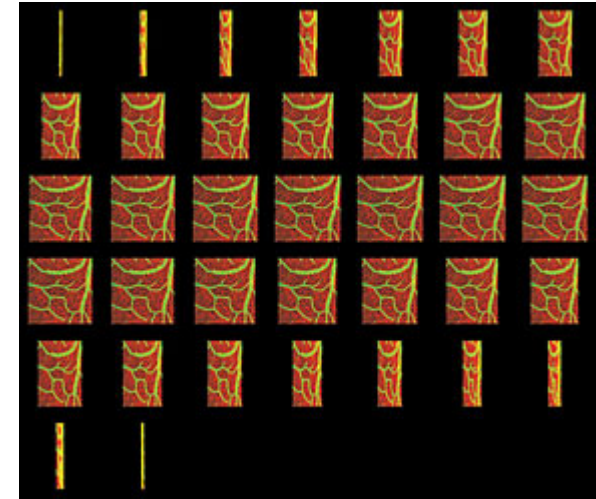
starting point: free floating section/tissue.

Ted Pella Biowave Pro

Retinas were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) overnight at 4°C. Following labeling the tissue was rinsed 6 x 20 minutes in buffer prior to beginning antibody labeling. The bench labeling protocol requires 7 days. These labeling results were completed in an afternoon using microwave-enhanced labeling (PELCO BioWave® with SteadyTemp™) during a workshop held at the Univ. of Minnesota Imaging Center (Mark Sanders, Director - May 17-19, 2006). The retinas were double-labeled for:

- Collagen Type IV (basal lamina surrounding blood vessels) with rabbit anti-type IV collagen and the secondary conjugated to FITC (green label)
- Glutamine Synthetase (enzyme found in retinal Müller glial cells) with mouse anti- glutamine synthetase and the secondary conjugated to Cy3 (red label)

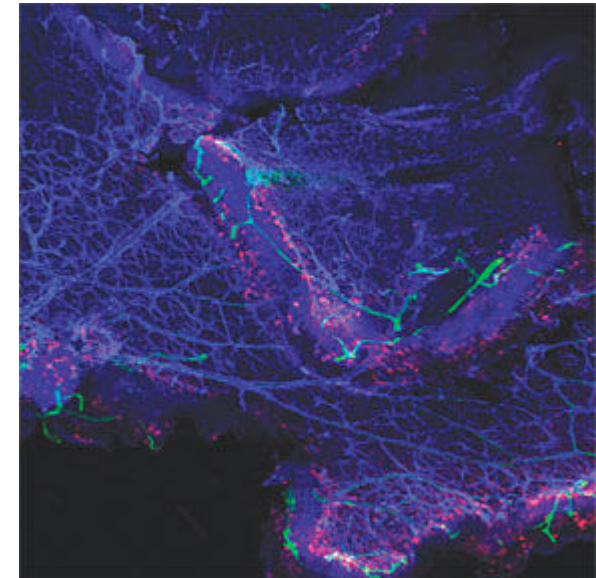
Primary antibody labeling was done at 170W for 12 minutes (4 on - 4 off - 4 on) under vacuum (15" Hg). Secondary antibody labeling was done at 170W for 6 minutes (2 on - 2 off - 2 on) under vacuum (15" Hg). Images were collected on a Nikon C1si Confocal Microscope.



Retinas were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) overnight at 4°C. Following fixation the tissue was rinsed 6 x 20 minutes in buffer prior to beginning antibody labeling. The bench staining protocol required 7 days. The labeling results were completed in an afternoon using microwave-enhanced labeling during a workshop held at the Univ. of Minnesota Imaging Center (Mark Sanders, Director - May 17-19, 2006). The retinas were triple-labeled for:

- Collagen Type IV (basal lamina surrounding blood vessels) with rabbit anti-type IV collagen and the secondary conjugated to FITC (green label)
- Glutamine Synthetase (enzyme found in retinal Müller glial cells) with mouse anti- glutamine synthetase and the secondary conjugated to Cy3 (red label)
- Glial Fibrillary acidic protein (GFAP an intermediate filament protein of astroglial cells) with chicken anti-glia fibrillary acidic protein and the secondary conjugated to Cy5 (blue label)

Primary antibody labeling was done at 170W for 12 minutes (4 on - 4 off - 4 on) under vacuum (15" Hg). Secondary antibody labeling was done at 170W for 6 minutes (2 on - 2 off - 2 on) under vacuum (15" Hg). Images were collected on a Nikon C1si Confocal Microscope.



Microwave Immunolabeling Protocol:

Ted Pella Biowave Pro

Start at the step that is appropriate for the labeling protocol being used.			
No.	Step	Microwave Settings Wattage/Temp Restriction	Time
1.	Coplin Jar - 50 ml. xylene - deparaffinization	250 W	4 min.
2.	Coplin Jar - 50 ml. 95% ethanol - rehydrate	250 W	1 min.
3.	Coplin Jar - Wash in tap water - rehydrate		30 sec.
4.	Antigen Retrieval (if required)	No specific method is recommended	
5.	Transfer Slides to Coverplate™ System ¹ Tissues to wellplates or petri dish		
6.	Block Endogenous Peroxidase - 3 drops (~100µl) Enzyme based chromogens	150-250 W	1 min.
7.	Buffer Rinse - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
8.	Blocking Step - 3 drops to ~1 ml Use Triton X or protease for thick sections >6µm	150-250 W	3 min. ²
9.	Primary Antibody - 3 drops (~100µl) Vacuum + increased time for thick tissues (>20µm)	150-250 W 15-20" Hg	6 min. ³ 12 min. ⁴
10.	Buffer Rinse - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
11.	Secondary Antibody - 3 drops (~100µl) Vacuum + increased time for thick tissues (>20µm)	150-250 W 15-20" Hg	6 min. ³ 12 min. ⁴
12.	Buffer Rinse - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
13.	Tertiary Attachment - 3 drops (~100µl) Vacuum + increased time for thick tissues (>20µm)	150-250 W 15-20" Hg	6 min. ³ 12 min. ⁴
14.	Buffer Rinse - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
15.	Chromagen - 100-500µl	150-250 W	1-6 min.
16.	Rinse in D.I. water - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
17.	Counter Stain - 100-500µl	150-250 W	1-3 min.
18.	Rinse in D.I. water - ~1 ml (1 to 3 x 1 min.)	150-250 W	1 min.
19.	Remove Slides from Coverplate™ System		
20.	Mount Coverslip and View		

¹The Coverplate™ System is described in Fig. 1

²3 min. corresponds to a preprogrammed time sequence (1 min. on-1 min. off-1 min. on)

³6 min. corresponds to a preprogrammed time sequence (2 min. on-2 min. off-2 min. on)

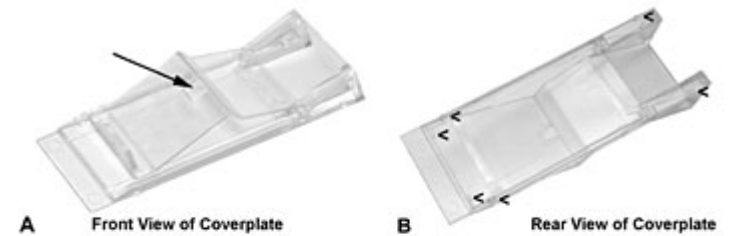
⁴12 min. corresponds to a preprogrammed time sequence (4 min. on-4 min. off-4 min. on)

Figure 1.

A. A front view of the Coverplate™ that is placed on the glass slide to form a capillary gap for the staining reagents to flow through. The arrow indicates the notch in the front clip of the Coverplate™ that connects with the tab in Fig. 1C (arrow).

B. The rear view of the Coverplate™ showing the tabs (<) that position and hold the slide (tissue side down) in position prior to placing the Coverplate™ and slide into the cassette base. Place approximately 2ml of buffer on the Coverplate™ prior to placement of the slide. Check to see that the capillary gap is completely filled with fluid (no bubbles). If bubbles are present rewet the surface and repeat slide placement.

C. The cassette base holds the Coverplate™ and slide complex together to maintain the capillary gap during microwave. The lid is not needed for microwave protocols



Cassette Base - Microwave Holder for Coverplate and Slide



Ted Pella Biowave Pro

The PELCO BioWave® Pro is newest model in our long history of microwave development. It has features that are unique to previous models and ideally suited for immunolabeling applications of all types. These features include:

1. Built-in magnetic stirrer for wellplates, petri dishes and PELCO PrepEze™ 6 and 12-well sample holders.
2. The ability to write protocols that preset parameters at each step and that direct the user through the labeling process; step-by-step.
3. New and improved PELCO ColdSpot® design for improved heat transfer and ease of use.
4. Automatic power calibration from low to high wattage in one single process.

State-of-the-art control is demonstrated by the degree of heating and uniformity of temperature between 2 types of wellplates (6 and 12-well) exposed to two different continuous wattage outputs for 1 minute.

630W of continuous power for 1 minute + 3x800ml water loads positioned in the microwave cavity to create a cold spot.

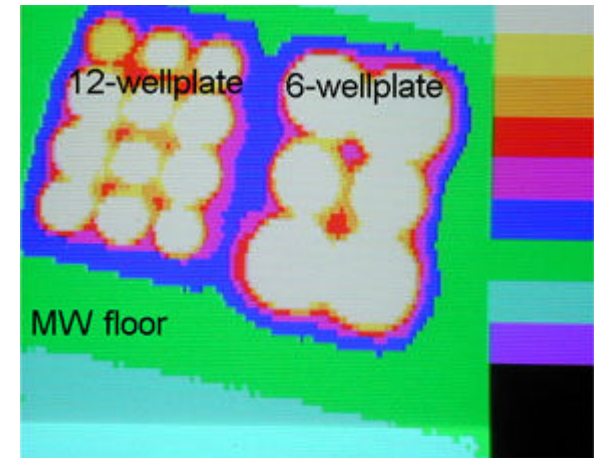
Temperature profile of 2 plastic wellplates after microwave exposure.

Starting temperature: 23°C

Ending temperatures: 6-well 32.7-42.5°C

12-well 32.3-35.4°C

Power output and heating profile typical of a standard laboratory microwave oven.



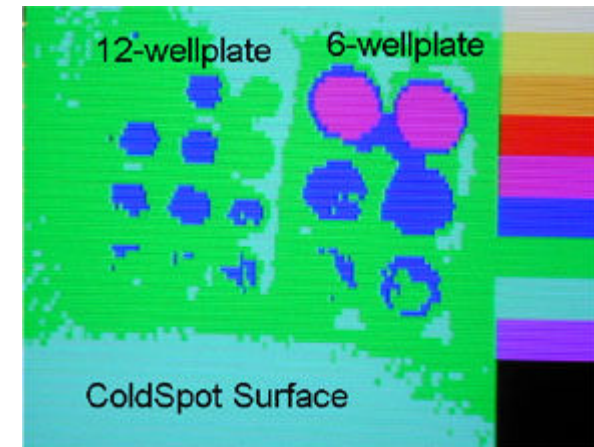
Ted Pella Biowave Pro

212W of continuous power for 1 minute + Patented PELCO ColdSpot® placed in the microwave cavity to create a large area of uniformity.

Temperature profile of 2 plastic wellplates after microwave exposure.

Starting temperature: 23°C
Ending temperatures: 6-well 25.3-26.5°C
12-well 23.3-25.3°C

Power output and heating profile typical of the PELCO BioWave® Pro set to the parameters best suited to immunolabeling .



Recent Literature

- Galvez, J.J., Adamson, G., Sanders, M.A., Giberson, R.T. (2006) Microwave tissue processing techniques: their evolution and understanding. *Microscopy and Analysis*, 20(6):23-24.
- Sanders, M.A, Anderson, T.E., Giberson, R. (2006) Microwave methods - evidence to support a microwave effect. *Microsc. Microanal.* 12(Suppl.2:Proceedings CD):295-296.
- Munoz, T.E., Giberson, R.T., Demaree, R., Day J.R. (2004) Microwave-assisted immunostaining: a new approach yields fast and consistent results. *J. Neurosci. Methods*, 137:133-139.
- Galvez, J.J., Giberson, R.T., Cardiff, R.D. (2004) Microwave mechanisms - the energy/heat dichotomy. *Microsc. Today*, 12(2):18-23.
- Giberson, R.T. (2002) The use of new microwave techniques to facilitate the immunostaining of paraffin sections on glass slides. *Microsc Microanal* 8(Suppl 2):162-163.
- Sanders, M.A. (2002) Recent Advances in microwave assisted specimen processing: keeping it cool. *Microsc. Microanal.* 8(Suppl.2):158-159.
- Sanders, M.A. and Gartner, D.M. (2001) In vivo microwave-assisted labeling of Allium and Drosophila nuclei. In: *Microwave Techniques and Protocols*. R.T. Giberson and R.S. Demaree, Jr. eds. Humana Press, Inc. Totowa, NJ. pp155-164.
- Rangell, L.K., Keller, G-A. (2000) Application of microwave technology to the processing and immunolabeling of plastic-embedded and cryosections. *J. Histochem. Cytochem.*, 48:1153-1159.

Ted Pella Biowave Pro