

Immunohistochemistry of Paraffin Embedded Tissues using the MicroProbe System

McAllister Lab, updated 09.21.09

MicroProbe Staining System: Fisher cat# 15-188-10

Reagents:

1. **IMPORTANT:** Paraffin embedded tissue sections must be mounted onto ProbeOn Plus microscope slides (Fisher cat# 22-230-900)
2. Dewaxing agent – Xylene (use under fume hood)
3. Redusol – Provided as 1x reagent (Genetex Immunostain Blocker, cat# GTX73323)
4. Automation Buffer – Stock is 10x (Genetex Immunostain Wash Buffer, cat# GTX73344). Dilute with H₂O to make 1x
5. Hydrogen Peroxide (1% vol/vol) in MeOH. Add 1.6 ml H₂O₂ in 50 ml MeOH. NOTE: This solution must be made fresh for each staining procedure
6. Secondary Antibody Kit – Vector Kits. Kits contain blocking serum, secondary antibody and detector complex (ABC). Be sure secondary antibody is directed against species in which primary antibody is generated. See kit instructions. (Vector Labs cat# PK6102 / Vector Labs cat# PK6101)
7. Peroxidase Enhancer (Invitrogen cat# P36931) – use per kit instructions
8. Chromogen Kit – Most applications work with peroxidase-based chromogens. AEC is best for aqueous conditions (Genetex cat# GTX82977)
9. Hematoxylin – Use aqueous hematoxylin (Vector Labs cat# H3404)
10. Mounting media – (Dako Faramount, Dako cat# S3025)
11. Dako Pen – To draw a water impermeable line around tissue section (Dako cat# S2002)

Procedure

1. Put sections in Microprobe slide holder by placing tissue section slide face-to-face with a clean ProbeOn Plus microscope slide. It is very important to only use this type of microscope slide – others will not work. Blank slides can be re-used – clean with water and ethanol after each use.
2. See following table for protocol

STEP	REAGENT	TIME (MIN)	TEMP	NOTES
1	None	5	60	
2	Dewaxing agent (xylene)	1	RT	
3	Dewaxing agent (xylene)	2	RT	
4	100% Ethanol	1 wash	RT	
5	Redusol	2	40	
6	Automation Buffer	1	RT	
7	Automation Buffer	4 washes	RT	
8	H ₂ O ₂ in MeOH (fresh)	2	40	
9	Automation Buffer	4 washes	RT	
10	Blocking serum (Vector kit)	5	40	
11	Automation Buffer	2 washes	RT	
12	Primary Antibody	15	40	
13	Automation Buffer	5 washes	RT	
14	Secondary Antibody (Vector kit)	15	40	
15	Automation Buffer	5 washes	RT	
16	Detector Reagent (ABC – kit)	10	40	
17	Automation Buffer	5 washes	RT	
18	Enhancer Reagent	1 wash	RT	
19	Chromogen	5	40	
20	Chromogen	10	40	
21	Distilled Water	2 washes	RT	
22	Take slides out of holder			
23	Hematoxylin	15-30 sec	RT	
24	Distilled Water	wash	RT	
25	Mount and coverslip			

Origin: Daugherty Lab, Wash Univ., July 7, 1996, SM