

## Helicobacter Pylori Antibody Protocol

1. Run slides to distilled water.
2. HIER using sodium citrate pH 6.0 solution in pressure cooker (steam) for 20 minutes.
3. Cool with lid off, power off 20 minutes.
4. Wash under cold running water, 10 minutes
5. Block with 3% hydrogen peroxide – 20 minutes
6. Wash – PBST – 2 X 5 minutes
7. Block with 2.5% appropriate blocking serum – 20 minutes
8. Without rinsing, shake off excess horse serum.
9. Primary antibody at 1:100 for at least 45 minutes.
10. Wash with PBST 2 X 5 minutes
11. Apply secondary antibody, anti-rabbit – 45 minutes
12. Wash in PBS 2 X 5 minutes
13. Prepare DAB brown (Vector DAB Brown).
14. Apply DAB to positive controls and watch under microscope – record time of positive control. To stop DAB reaction, use distilled water.
15. Wash in distilled water 5 minutes.
16. Pass Hematoxylin solution through 0.2 mm filter
17. Repeatedly dip slides in Hematoxylin for 30 seconds
18. Rinse with water by dipping in water bath for 10 seconds
19. Submerge in bath of 0.1% sodium bicarbonate solution for 30 seconds
20. Rinse with water by dipping in water bath for 5 seconds
21. Submerge slides in running water for 5 minutes
22. Dehydrate in 2 changes of 95% alcohol, 100% alcohol and clear in 2 changes of xylene.
23. Cover slip

Results:

Bacteria – Brown

Background – Blue

