



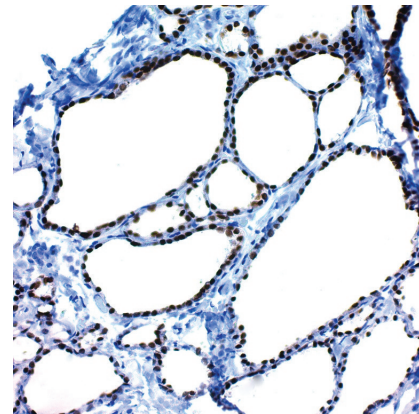
Instructions For Use CPH080-IFU		
Rev. Date: Nov. 1, 2017	Revision: 2	Page 1 of 3

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

CRF™ Anti-Polyvalent HRP Polymer (DAB) Stain Kit

Description: The CRF™ Anti-Polyvalent HRP Polymer (DAB) Stain Kit based on proprietary CRF™ Technology has been developed to provide the cleanest, most consistent staining available. Developed in the research laboratories of ScyTek, the system utilizes a polymerized peroxidase label that eliminates biotin and its' associated background issues from the equation. In addition, this product reduces the steps required for immunohistochemical staining by combining two steps from the traditional Biotin-Streptavidin system. The CRF™ technology based Anti-Polyvalent system is effective with antibodies of mouse, rat, rabbit and guinea pig.

Uses/Limitations: Not to be taken internally.
 For In Vitro Diagnostic Use.
 Histological applications.
 Do not use if reagents become cloudy.
 Do not use past expiration date.
 Use caution when handling reagents.
 Non-Sterile.



Control Tissue: Any well-fixed tissue section.
 Frozen tissue section.
 Cytocentrifuge preparation.

Ordering Information and Current Pricing at www.scytek.com

Test Capacity: 80 Slides


Kit Contents:	Item #	Description	Volume
	ADA008	Peroxide Block for Image Analysis	8 ml
	AAA008	Super Block	8 ml
	ABZ008	CRF™ Anti-Polyvalent HRP	8 ml
	ACB003	DAB Chromogen Concentrate	3 ml
	ACU005	DAB Substrate (High Contrast)	5 ml x 8 vials


Recommended, But Not Included:

Item #	Description
CPL500	Citrate Plus
HAQ500	Hematoxylin for Automation
BRT500	Bluing Reagent


Storage: Store at 2-8°C.

Precautions: Avoid contact with skin and eyes.
 Harmful if swallowed.
 Follow all Federal, State, and local regulations regarding disposal.

Storage: 2° C  8° C

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 Logan, UT 84321
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 2514 AP The Hague, The Netherlands

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Procedure:

1. Rehydrate tissue slides.
2. In a glass or plastic (Autoclavable) Coplin jar, add 5 ml of Citrate Plus (CPL) and 45 ml of deionized water.
3. Submerge slides in diluted Citrate Plus and loosely cap.
4. Add Distilled water to bottom of Autoclave or Pressure Cooker (about 1 inch deep in Pressure Cooker).
5. Place Coplin jar in Pressure Cooker or Autoclave.
6. Turn heat on and allow pressure to rise to 20-25 PSI.
7. Maintain pressure at 20-25 PSI for 5 minutes.
8. Turn off heat source and allow to cool.
9. When pressure has dropped to ambient, carefully remove lid or open door.
10. Using tongs, remove Coplin Jar and place on counter.
11. Once Coplin Jar cools to room temperature remove slides, rinse several times in buffer and proceed with staining as usual.
12. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes.
13. Rinse 3 times in buffer.
14. Apply Super Block (AAA), and incubate for 5 minutes at room temperature to block nonspecific background staining. **Note:** Do not exceed 10 minutes or there may be a reduction in desired stain.
15. Rinse 3 times in buffer.
16. Apply primary antibody and incubate according to manufacturer's protocol.
17. Rinse 3 times in buffer.
18. Apply CRF™ Anti-Polyvalent HRP and incubate for 30 minutes at room temperature.
19. Rinse 3 times in buffer.

WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

20. Add 1 drop (40-50ul) DAB Chromogen (ACB) to each 1 ml of DAB Substrate (High Contrast) (ACU), mix by swirling and apply to tissue for 5 minutes.
21. Rinse 1 time in DI Water.
22. Apply DAB Chromogen/Substrate mixture and incubate for a second 5 minute period.
23. Rinse 3 times in buffer.
24. Apply Hematoxylin for Automation (HAQ) and incubate for 1 minute.
25. Rinse 3 times in distilled water.
26. Apply Bluing Reagent (BRT) and incubate for 5-10 seconds.
27. Rinse immediately in distilled or deionized water.
28. Dehydrate slides and clear in xylene or xylene substitute.
29. Coverslip using a permanent mounting media.

Storage: 2° C



8° C



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-Troubleshooting Guide-

Overstaining:

1. Concentration of the primary antibody was too high or the incubation time was too long.
2. Temperature during incubation was too high.
3. Incubation times were too long.

Non-Specific Background Staining:

1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Antigen migrated in tissue.
5. Excessive tissue adhesive on slides.
6. Inadequate blocking with protein block.

Weak Staining:

1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents are past their expiration date.
3. Inadequate removal of wash buffer between steps, resulting in dilution of reagents.
4. Room temperature was excessively cool.
5. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
6. Excessive incubation with protein block (Super Block or normal serum).

No Staining:

1. Steps were inadvertently left out.
2. There is no antigen in the tissue.
3. The primary antibody is not of mouse, rat, rabbit or guinea pig origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.
5. One or more components of the kit have been inactivated.

References:

1. Duncan SM, Seigel GM. High-contrast enzymatic immunohistochemistry of pigmented tissues. *Journal of Biological Methods*. 2016 Jul 12;3(3):e47.
2. Das P, Gahlot GP, Mehta R, Makharia A, Verma AK, Sreenivas V, Panda SK, Ahuja V, Gupta SD, Makharia GK. Patients with mild enteropathy have apoptotic injury of enterocytes similar to that in advanced enteropathy in celiac disease. *Digestive and Liver Disease*. 2016 Jun 21.
3. Seigel GM, Sharma S, Hackam AS, Shah DK. HER2/ERBB2 immunoreactivity in human retinoblastoma. *Tumor Biology*. 2015 Nov 27:1-8.

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