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) Lab Pack

Description: The CRF™ Anti-Polyvalent HRP Polymer (DAB) Lab Pack 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 only.
Do not use if reagent becomes cloudy.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile.

REF# **CPP125**

Test Capacity: Up to 1250 Slides

Contents:

Item #	Description	Volume
AAA125	Super Block	125 ml
ABZ125	CRF™ Anti-Polyvalent HRP	125 ml
ACB008	DAB Chromogen Concentrate	8 ml
ACU125	DAB Substrate (High Contrast)	125 ml

REF# **CPP500**

Test Capacity: Up to 5000 Slides

Contents:

Item #	Description	Volume
AAA500	Super Block	500 ml
ABZ500	CRF™ Anti-Polyvalent HRP	500 ml
ACB030	DAB Chromogen Concentrate	30 ml
ACU500	DAB Substrate (High Contrast)	500 ml

REF# **CPP999**

Test Capacity: Up to 10000 Slides

Contents:

Item #	Description	Volume
AAA999	Super Block	1000 ml
ABZ999	CRF™ Anti-Polyvalent HRP	1000 ml
ACB060	DAB Chromogen Concentrate	60 ml
ACU999	DAB Substrate (High Contrast)	1000 ml

Storage: 2° C  8° C

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Recommended, But Not Included:

Item #	Description
CPL500	Citrate Plus
ADA500	Peroxide Block for Image Analysis
HMM500	Hematoxylin, Mayer's (Lillie's Modification)
BRT500	Bluing Reagent

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

Procedure:

1. Rehydrate tissue slides.
2. Recommended Procedure: Perform retrieval procedure according to protocol of reagent used (Citrate Plus cat# CPL500).
3. After retrieval, proceed with staining as usual.
4. Apply Peroxide Block for Image Analysis (ADA) and incubate slide for 10-15 minutes.
5. Rinse 3 times in buffer.
6. 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.
7. Rinse 3 times in buffer.
8. Apply primary antibody and incubate according to manufacturer's protocol.
9. Rinse 3 times in buffer.
10. Apply CRF™ Anti-Polyvalent HRP Polymer and incubate for 30 minutes at room temperature.
11. Rinse 3 times in buffer.
12. Rinse 1 time in Distilled/DI water.

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

13. Add 50ul of DAB Chromogen Concentrate (ACB) to each 1ml vial of DAB Substrate High Contrast, mix by swirling and apply to tissue for 5 minutes.
14. Rinse 1 time in Distilled/DI water.
15. Apply DAB Chromogen/Substrate mixture and incubate for a second 5 minute period.
16. Rinse 3 times in buffer.
17. Apply Hematoxylin , Mayer's (HMM) and incubate for 5 minutes.
18. Rinse 3 times in distilled water.
19. Apply Bluing Reagent (BRT) and incubate for 5 seconds.
20. Rinse immediately in distilled or deionized water.
21. Dehydrate slides and clear in xylene or xylene substitute.
22. 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.