Store at 4°C

SignalStain® IHC Dual Staining Kit (HRP, Rabbit, Brown / AP, Mouse, Red)

#82456

1 Kit
(120 slides)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

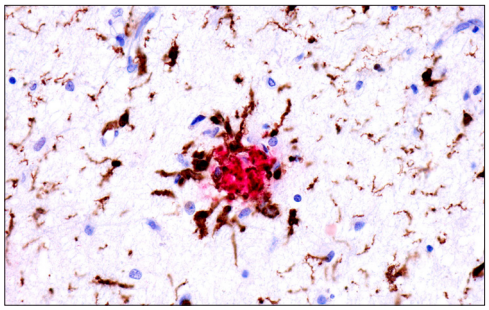
Product Includes	Item #	Kit Quantity
SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)	8114	15 mL
SignalStain® Boost IHC Detection Reagent (AP, Mouse)	31926	15 mL
SignalStain® DAB Substrate Kit	8059	1 kit
SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit	76713	1 kit

Description: The SignalStain® IHC Dual Staining Kit (HRP, Rabbit, Brown / AP, Mouse, Red) provides reagents needed to perform enzymatic labeling of two different target antigens on the same tissue section using antibodies derived from rabbit and mouse. The reagents in this kit are thoroughly validated using our IHC-recommended antibodies and will perform optimally when the two target antigens are expressed in different cell compartments or different cell types. This kit includes sufficient reagents for 120 slides based on a 100 µL assay volume. All reagents in this kit are available individually.

IMPORTANT: The SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) is paired with the SignalStain® DAB Substrate Kit and the SignalStain® Boost IHC Detection Reagent (AP, Mouse) is paired with the SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit for detection.

DAB is a suspected carcinogen. Good laboratory practices should be followed, and appropriate protective equipment should be used when handling DAB, including gloves, protective eyewear, and a lab coat. Dispose of in accordance with local regulations.

Appropriate personal protective equipment should be worn when handling Vibrant Red. Dispose of in accordance with local regulations.



Dual immunohistochemical analysis of paraffin-embedded human Alzheimer's brain using Iba1/AIF-1 (E404W) XP® Rabbit mAb #17198 (brown) and APP/β-Amyloid (NAB228) Mouse mAb #2450 (red).

Storage: All components in this kit are stable for at least 12 months past the reference date indicated on the component label when stored at 4°C and left unused.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Directions for Use: Before executing a dual staining experiment, it is suggested to optimize each primary antibody for a 1 hour primary incubation. If the recommended antigen retrieval buffer is not the same for each antibody, then it is suggested to use EDTA for both antibodies.

If the primary antibodies are not compatible with the same antibody diluent, then it is advised to perform the primary incubation and detection steps sequentially.

After determination of the appropriate antibody dilution, it is recommended to test the order of chromogen development. There may be differences in the quality of the results depending upon which chromogen is developed first. This is accomplished by combining the primary antibodies on two slides for the primary incubation. One slide should have DAB developed first, followed by Vibrant Red. The other slide should have Vibrant Red developed first, followed by DAB. Review the slides and determine which order of chromogens yields optimal results.

It is possible that an antibody's performance will not be the same with all chromogens. If during the course of assay optimization it is determined that an antibody is not yielding ideal results, it is advised to test an alternate detection reagent/chromogen pair (e.g., SignalStain® IHC Dual Staining Kit (AP, Rabbit, Red / HRP, Mouse, Brown) #36084).

Staining Procedure:

1. For paraffin-embedded sections, deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
2. Perform antigen retrieval as recommended by the primary antibody manufacturer. If the recommended antigen retrieval buffer is not the same for each antibody, then it is suggested to use EDTA for both antibodies.
3. After washing, incubate sections in 3% H_2O_2 diluted in water for 10 minutes.
4. Wash sections in dH2O two times for 5 minutes each.
5. Incubate sections for 30 minutes with blocking solution.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

6. Remove blocking solution from sections and incubate with rabbit and mouse primary antibodies diluted in the appropriate antibody diluent as recommended by the primary antibody manufacturer. If the primary antibodies are not compatible with the same antibody diluent, then it is advised to perform the primary incubation and detection steps sequentially.
7. Wash.
8. Incubate for 30 minutes with either the AP or HRP Boost Detection Reagent previously determined to be first.
9. Wash.
10. Apply the AP or HRP-compatible substrate, matching the Boost Detection Reagent used first, and develop for the recommended time. See Substrate Reagent Preparation below.
11. Wash.
12. Incubate for 30 minutes with either the AP or HRP Boost Detection Reagent previously determined to be second.
13. Wash.
14. Apply the AP or HRP-compatible substrate, matching the Boost Detection Reagent used second, and develop for the recommended time. See Substrate Reagent Preparation below.
15. Wash.
16. Counterstain (optional), then clear and mount coverslips.

Substrate Reagent Preparation:

SignalStain® DAB Substrate Kit #8059:

1. To prepare the DAB working solution, add 30 µL SignalStain® DAB Chromogen Concentrate to 1 mL SignalStain® DAB Diluent. Mix well before use. Working solutions are stable for up to 14 days when stored at 4ºC or up to 5 days when stored at room temperature (approximately 25ºC). While the solution may change color over that time period, it will have no effect on the quality of the staining.
2. After incubation with SignalStain® Boost IHC Detection Reagent or other HRP-based detection systems, wash slides and incubate with the working solution at room temperature until staining develops. 1-10 minutes generally provides an acceptable staining intensity.

SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit #76713:

1. To prepare the Vibrant Red working solution, to 5 mL SignalStain® Vibrant Red Diluent add 130 µL SignalStain® Vibrant Red Reagent 1 and 50 µL SignalStain® Vibrant Red Reagent.
2. Mix well before use. Working solutions should be used within 15 minutes of preparation.
   IMPORTANT: Do not heat Vibrant Red kit components or working solution.
3. After incubation with SignalStain® Boost IHC Detection Reagent, wash slides and incubate with the working solution at room temperature for 20-30 minutes.