Store at -20°C

SignalStar™ Fluorescence Removal Kit

#32722

1 Kit
(10 slides)

Support: +1-978-867-2388 (U.S.)
cellsignal.com/support

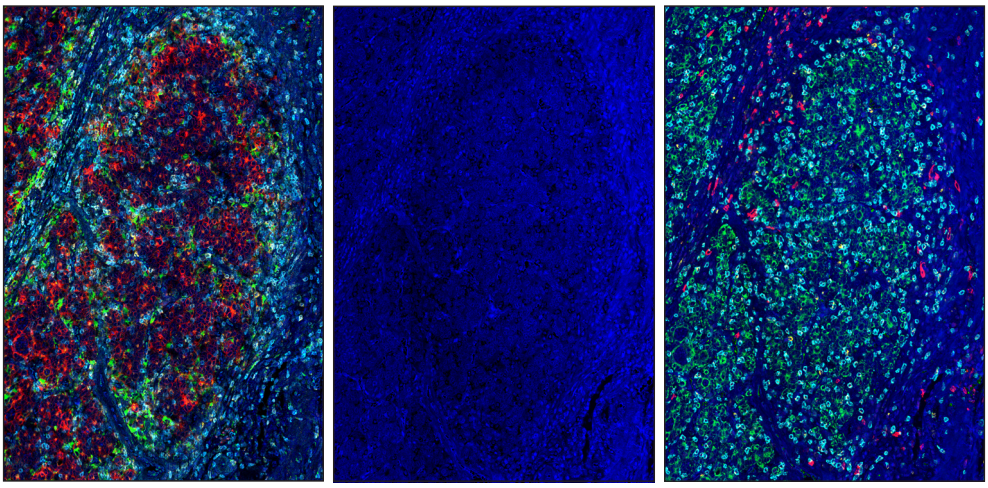
Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

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

Product Includes	Item #	Kit Quantity	Storage Temp
10X dsDNase Buffer	55078	1 x 370 µL	-20°C
dsDNase	73805	1 x 37 µL	-20°C

Description: SignalStar multiplex immunohistochemistry (IHC) is an advanced technology for labeling multiple proteins simultaneously in tissue samples using specific primary antibodies and fluorescent detection reagents. This technology offers accuracy and reliability in visualizing and analyzing protein expression while maintaining spatial context and tissue architecture.

The SignalStar Fluorescence Removal Kit is compatible with SignalStar Oligo-Antibody Pairs, SignalStar Midplex IHC Buffer Kit #29414, and SignalStar Miniplex IHC Buffer Kit #63043 for use in fluorescent multiplex imaging experiments. This product is required to remove fluorescent oligos following the final round of signal amplification and imaging of SignalStar Oligo-Antibody Pairs. This buffer kit includes the reagents required for 10 slides. SignalStar Multiplex IHC Kits & Reagents are not compatible with all of Cell Signaling Technology® products and protocols that are recommended for use in immunohistochemical assays.



SignalStar multiplex immunohistochemical analysis of paraffin-embedded human gastric adenocarcinoma using CD68 (D4B9C) & CO-0007-488 SignalStar™ Oligo-Antibody Pair #73071 (green), PD-1 (Intracellular Domain) (D4W2J) & CO-0008-594 SignalStar™ Oligo-Antibody Pair #35347 (yellow), PD-L1 (E1L3N®) & CO-0005-647 SignalStar™ Oligo-Antibody Pair #52085 (red), CD3ε (D7A6E) & CO-0001-750 SignalStar™ Oligo-Antibody Pair #51754 (cyan), and DAPI #4083 (blue) in Imaging Round 1 (left). Following Imaging Round 1, SignalStar oligonucleotides and fluorophores were removed, and the tissue was imaged again using DAPI #4083 (middle, blue). Subsequently, a second round of SignalStar multiplex immunohistochemical analysis was performed using Pan-Keratin (C11) & CO-0003-488 SignalStar™ Oligo-Antibody Pair #63566 (green), Granzyme B (D6E9W) & CO-0009-594 SignalStar™ Oligo-Antibody Pair #15194 (yellow), CD20 (E7B7T) & CO-0011-647 SignalStar™ Oligo-Antibody Pair #36775 (red), CD8α (D8A8Y) & CO-0004-750 SignalStar™ Oligo-Antibody Pair #62750 (cyan), and DAPI #4083 (blue) in Imaging Round 2 (right). All fluorophores have been assigned a pseudocolor, as indicated. Staining was performed on the BOND RX autostainer by Leica Biosystems.

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature.

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

Reagents Not Supplied:

- SignalStar™ Oligo-Antibody Pairs
- SignalStar™ Midplex IHC Buffer Kit #29414
- SignalStar™ Miniplex IHC Buffer Kit #63043
- Nuclease-free Water #12931

Directions for Use: The SignalStar Fluorescence Removal Kit is intended for use following the SignalStar™ Multiplex IHC Assay Manual protocol or the SignalStar™ Multiplex IHC Assay for Use on BOND RX Fully Automated Research Stainer by Leica Biosystems protocol. Continue to follow all guidance and recommendations from the original protocol when using the SignalStar Fluorescence Removal Kit.

For the SignalStar™ Multiplex IHC Assay Manual protocol:

1. After the second round of image acquisition, soak slides in dH_2O for >30 min to gently remove coverslip.
2. Combine reagents to create the SignalStar Fluorescence Removal Solution:
   a. For 5 slides: 75 µL 10X dsDNase Buffer, 7.5 µL dsDNase, and 667.5 µL Nuclease-free Water #12931.
   b. For 10 slides: 150 µL 10X dsDNase Buffer, 15 µL dsDNase, and 1,335 µL Nuclease-free Water #12931.
3. Cover with parafilm and vortex for 10 sec. Store all SignalStar kit components on ice when preparing solutions. SignalStar solutions should be used promptly once all reagents have been combined for the run.
4. Incubate slides in 150 µL of SignalStar Fluorescence Removal Solution for 2 hr at 37°C. (Use of a covered humidity chamber is highly recommended to prevent evaporation.)
5. Immerse slides in dH_2O for 30 sec.
6. Complete any additional staining and imaging steps following your manufacturer's protocol.

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.

For the SignalStar™ Multiplex IHC Assay for Use on BOND RX Fully Automated Research Stainer by Leica Biosystems protocol:

1. In the BOND RX software by Leica Biosystems, create a copy of the "CST SignalStar Imaging Round 2" protocol.
2. Change the name of the copy to "CST SignalStar Imaging Round 3" with the abbreviated name "CST Rd3."
3. Select "Show wash steps."
4. Delete Steps 8-63, saving only steps 1-7 for fluorescence removal.
5. Add steps required for any additional staining following your manufacturer's protocol.
6. Select "CST SignalStar" as the preferred Detection System.

   Note: Additional staining steps may require further selection of other Detection Systems. Please contact Leica Biosystems for assistance.
7. Select "Create Protocol."
8. Click the Save button and then the Yes button to acknowledge the caution message.
9. After the second round of image acquisition, soak slides in dH_2O for >30 min to gently remove coverslip.
10. Combine reagents to create the SignalStar Fluorescence Removal Solution:
    a. For 5 slides: 185 µL 10X dsDNase Buffer, 18.5 µL dsDNase, and 1,646.5 µL Nuclease-free Water #12931 in 1 BOND Titration Insert.
    b. For 10 slides: 335 µL 10X dsDNase Buffer, 33.5 µL dsDNase, and 2,981.5 µL Nuclease-free Water #12931 in 1 BOND Titration Insert.
11. Cover with parafilm and vortex for 10 sec. Store all SignalStar kit components on ice when preparing solutions. SignalStar solutions should be used promptly once all reagents have been combined for the run.
12. In the BOND RX software, create a study and add slides.
13. When "adding slides," use the selections below for Tissue Preparation on BOND:
    a. Slide preparation: -- (no value/not required)
    b. Dispense Volume: 150 µL
    c. HIER: -- (no value/not required)
14. Select "CST SignalStar Imaging Round 3," ensuring that Slide preparation is selected as "--" and HIER is selected as "--."
15. Print labels, add labels to slides, and place slides onto the slide tray.
16. Place 2-3 drops of dH_2O onto each slide before adding BOND covertiles.
17. Start the BOND RX run.