#8109 Store at 4°C and -20°C

SignalStain® Proliferation/Apoptosis IHC Sampler Kit

☑ 1 Kit

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Antigen Retrieval/Diluent	Isotype
Cleaved Caspase-3 (Asp175) Antibody	9661	40 µl	Citrate/SignalStain® Antibody Diluent #8112	Rabbit IgG
Phospho-Histone H3 (Ser10) Antibody	9701	40 µl	Citrate/SignalStain® Antibody Diluent #8112	Rabbit IgG
PCNA (PC10) Mouse mAb	2586	40 µl	Citrate/SignalStain® Antibody Diluent #8112	Mouse IgG2a
Survivin (71G4B7E) Rabbit mAb	2808	40 µl	Citrate/SignalStain® Antibody Diluent #8112	Rabbit IgG
*SignalStain® Antibody Diluent	8112	25 ml		
*SignalSlide® Cleaved Caspase-3 (Asp175) IHC Controls	8104	1 Pack		

See www.cellsignal.com for individual component applications, species cross-reactivity, and additional application protocols.

Description: The SignalStain® Proliferation/Apoptosis IHC Sampler Kit from Cell Signaling Technology allows the researcher to examine paraffin-embedded tissues or cells with antibodies that will detect cellular apoptosis or proliferation. Each antibody is validated for use in immunohistochemical assays using multiple approaches. Also included in the kit are control slides that can be used to verify the performance of each antibody and a primary antibody diluent. Please see table above for recommended diluent for each antibody in this kit.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments (2).

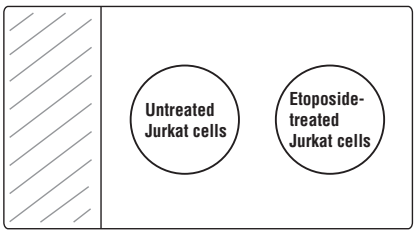
Survivin is a 16 kDa anti-apoptotic protein highly expressed during fetal development and cancer cell malignancy (3). Survivin binds and inhibits caspase-3, controlling the checkpoint in the G2/M-phase of the cell cycle through inhibiting apoptosis and promoting cell division (4,5).

Proliferating cell nuclear antigen (PCNA) is a member of the DNA sliding clamp family of proteins that assist in DNA replication (6). Multiple proteins involved in DNA replication, DNA repair, and cell cycle control bind to PCNA rather than directly associating with DNA, thus facilitating fast processing of DNA (reviewed in 7). PCNA protein expression is a well-accepted marker of proliferation, and PCNA (PC10) Mouse mAb has been used to assess PCNA levels in hundreds of scientific studies.

Histone H3, H2A, H2B, and H4 make up the core histones of the nucleosome. In response to various stimuli, the amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination, which affect the accessibility of chromatin to transcription factors (8). Phosphorylation of Histone H3 at Ser10, Ser28 and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (9-11).

Specificity/Sensitivity: Each antibody in the SignalStain® Proliferation/Apoptosis IHC Sampler Kit detects endogenous levels of its target protein and does not cross-react with any related proteins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a recombinant Protein A-PCNA fusion protein, or with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Cys60 of human Survivin. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser10 of human histone H3, or with a synthetic peptide surrounding amino-terminal residues adjacent to Asp175 of human caspase-3. Antibodies are purified by protein A and peptide affinity chromatography.



Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C.

*SignalStain® Antibody diluent is supplied as a working solution and should be stored at 4°C (packaged separately).

*Control slides should be stored at 4°C (packaged separately).

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:

Phospho-Histone H3 (Ser10) Antibody ***#9701***
Immunohistochemistry (Paraffin) 1:100
IHC Protocol: Unmasking buffer/Antibody diluent Citrate/SignalStain® Antibody Diluent #8112
Immunohistochemistry (Frozen) 1:100
Fixative: 10% Neutral buffered formalin

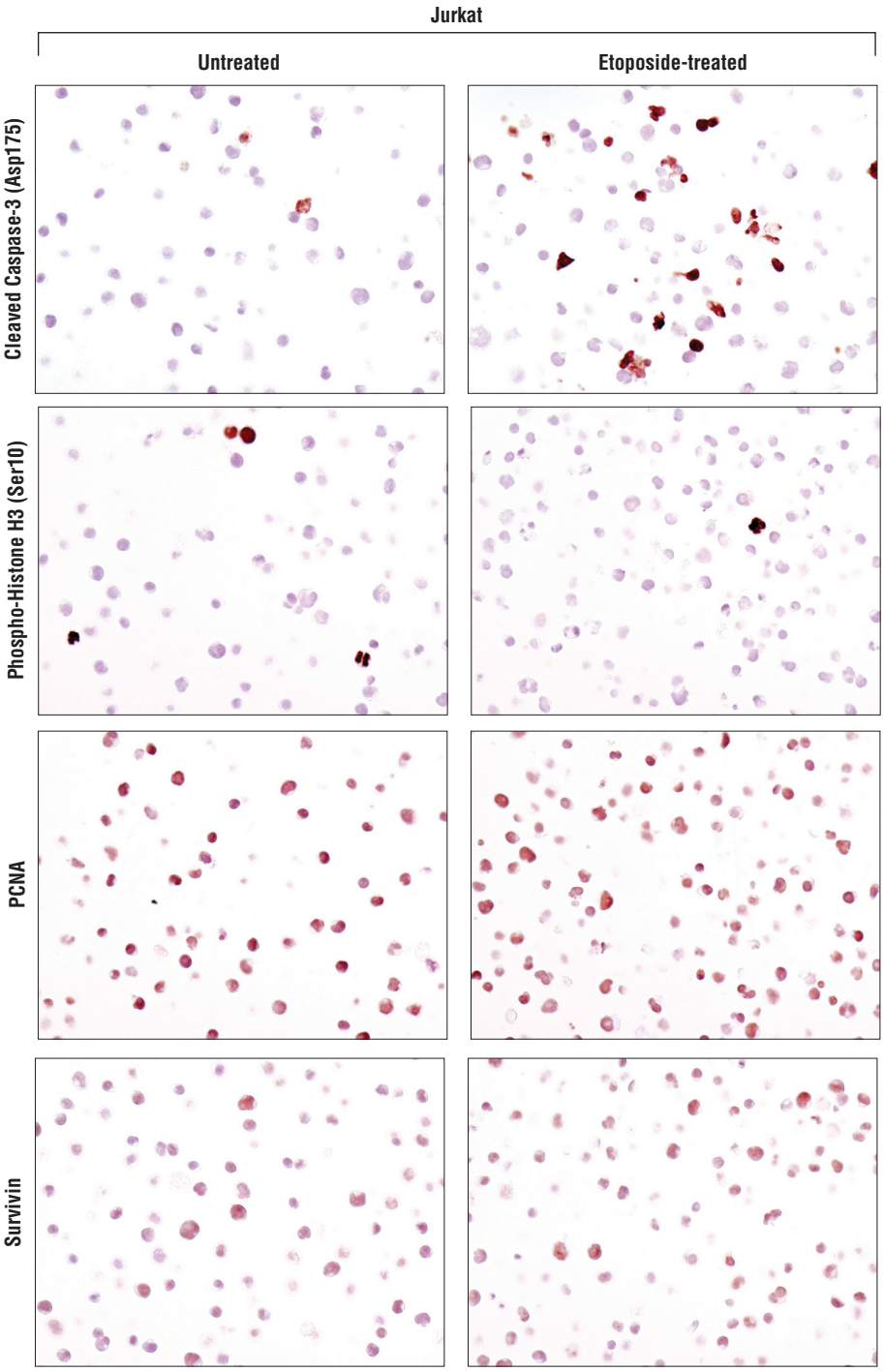
PCNA (PC10) Mouse mAb ***#2586***
Immunohistochemistry (Paraffin) 1:4000
IHC Protocol: Unmasking buffer/Antibody diluent Citrate/SignalStain® Antibody Diluent #8112

Survivin (71G4) Rabbit mAb ***#2808***
Immunohistochemistry (Paraffin) 1:400
IHC Protocol: Unmasking buffer/Antibody diluent Citrate/SignalStain® Antibody Diluent #8112
Immunohistochemistry (Frozen) 1:400
Fixative: 10% Neutral buffered formalin

Cleaved Caspase-3 (Asp175) Antibody ***#9661***
Immunohistochemistry (Paraffin) 1:400
IHC Protocol: Unmasking buffer/Antibody diluent Citrate/SignalStain® Antibody Diluent #8112
Immunohistochemistry (Frozen) 1:200
Fixative: 3% formaldehyde

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Immunohistochemical analysis of paraffin embedded Jurkat cell pellets, untreated (left) or etoposide-treated (right), using Phospho-Histone H3 (Ser10) Antibody, PCNA (PC10) Mouse mAb, Cleaved Caspase-3 (Asp175) Antibody and Survivin (71G4B7E) Rabbit mAb. Cell pellets are provided in the SignalSlide® Cleaved Caspase-3 (Asp175) IHC Controls.

Immunohistochemistry Protocol (Paraffin)

***IMPORTANT:** See product data sheet for the appropriate antibody diluent and antigen unmasking procedure. **IHC Protocol:** Unmasking buffer/antibody diluent.

A Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH_2O)
4. Hematoxylin (optional)
5. **Wash Buffer: 1X TBS/0.1% Tween®20 (1X TBST):** To prepare 1 L add 100 ml 10X TBS to 900 ml dH_2O. Add 1 ml Tween®20 and mix.
   10X Tris Buffered Saline (TBS): To prepare 1 L add 24.2 g Trizma® base ($C_4H_{11}NO_3$) and 80 g sodium chloride (NaCl) to 1 L dH_2O. Adjust pH to 7.6 with concentrated HCl.
6. ***Antibody Diluent:**
   a. **SignalStain® Antibody Diluent** #8112
   b. **TBST/5% normal goat serum:** To 5 ml 1X TBST add 250 µl normal goat serum.
   c. **PBST/5% normal goat serum:** To 5 ml 1X PBST add 250 µl normal goat serum.

   1X PBS/0.1% Tween®20 (1X PBST): To prepare 1L add 100 mL 10X PBS to 900 mL dH_2O. Add 1 ml Tween®20 and mix.

   10X Phosphate Buffered Saline (PBS): To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 7.4.
7. ***Antigen Unmasking:**
   a. **Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.
   b. **EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 8.0.
   c. **TE:** 10 mM Tris/1 mM EDTA/0.05% Tween–20, pH 9.0: To prepare 1L add 1.21 g Trizma® base ($C_4H_{11}NO_3$) and 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2 \bullet 2H_2O$) to 950 ml dH_2O. Adjust pH to 9.0, add 0.5 ml Tween–20, then adjust final volume to 1000 ml with dH_2O.
   d. **Pepsin:** 1 mg/ml in Tris-HCl pH 2.0.
8. **3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H_2O_2 to 90 ml dH_2O.
9. **Blocking Solution:** TBST/5% normal goat serum: to 5ml 1X TBST add 250 µl normal goat serum.
10. Biotinylated secondary antibody.
11. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
12. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Deparaffinization/Rehydration

NOTE: Do not allow slides to dry at any time during this procedure.

1. **Deparaffinize/hydrate sections:**
   a. Incubate sections in three washes of xylene for 5 minutes each.
   b. Incubate sections in two washes of 100% ethanol for 10 minutes each.
   c. Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH_2O for 5 minutes each.

C *Antigen Unmasking

NOTE: Consult product data sheet for specific recommendation for the unmasking solution.

1. **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
2. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.
3. **For TE:** Bring slides to a boil in 10 mM TE/1 mM EDTA/0.05% Tween®20, pH 9.0 then maintain at a sub-boiling temperature for 18 minutes. Cool on the bench for 30 minutes.
4. **For Pepsin:** Digest for 10 minutes at 37°C.

D Staining

1. Wash sections in dH_2O three times for 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections in dH_2O twice for 5 minutes each.

NOTE: Consult product data sheet for recommended antibody diluent.

4. Wash section in wash buffer for 5 minutes.
5. Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100-400 µl primary antibody diluted in recommended antibody diluent to each section. Incubate overnight at 4°C.
7. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
8. Add 100-400 µl biotinylated secondary antibody, diluted in TBST per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
9. If using ABC avidin/biotin method, prepare ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
10. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
11. Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
12. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
13. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
14. As soon as the sections develop, immerse slides in dH_2O.
15. If desired, counterstain sections in hematoxylin per manufacturer's instructions.
16. Wash sections in dH_2O two times for 5 minutes each.
17. Dehydrate sections:
    a. Incubate sections in 95% ethanol two times for 10 seconds each.
    b. Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    c. Repeat in xylene, incubating sections two times for 10 seconds each.
18. Mount coverslips.

Immunohistochemistry Frozen Section Protocol

A Solutions and Reagents

1. Xylene,
2. Ethanol (anhydrous denatured, histological grade 100% and 95%)
3. Hematoxylin (optional)
4. **Fixative: For optimal fixative, please refer to the product data sheet**,
   - **4a.** 10% neutral buffered formalin
   - **4b.** Acetone
   - **4c.** Methanol
   - **4d.** 16% formaldehyde
     - **4d1. 3% formaldehyde:** To prepare, add 18.75 ml 16% formaldehyde to 81.25 ml 1X TBS.
5. **10X Tris Buffered Saline (TBS):** To Prepare 1 L add 24.2 g Trizma base ($C_4H_{11}NO_3$) and 80 g sodium chloride (NaCl) to 1 L dH_2O. Adjust pH to 7.6 with concentrated HCl.
6. **Wash buffer:** 1X Tris Buffered Saline (TBS) To prepare 1 L add 100 ml 10X TBS to 900 ml dH_2O.
7. **Methanol/Peroxidase:** To prepare, add 10 mL 30% H_2O_2 to 90 ml methanol. Store at -20°C.
8. **Blocking Solution:** 1X TBS/0.3% Triton-X 100/5% normal goat serum
   To prepare: add 500 µl goat serum and 30 µl Triton-X 100 to 9.5 ml 1X TBS.
9. **Biotinylated Secondary Antibody.**
10. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA). Prepare according to manufacturer's instructions 30 minutes before use.
11. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Sectioning

1. **For tissue stored at -80°C:** remove from freezer and equilibrate at -20°C for approximately 15 minutes before attempting to section. This may prevent cracking of the block when sectioning.
2. Section tissue at a range of 6-8 µm and place on positively charged slides.
3. Allow sections to air dry on bench for a few minutes before fixing (this helps sections adhere to slides).

C Fixation

NOTE: Consult product data sheet to determine the optimal fixative.

1. After sections have dried on the slide, fix in optimal fixative as directed below.
   - **1a. 10% Neutral buffered formalin:** 10 minutes at room temperature. Proceed with staining procedure immediately.
   - **1b. Cold acetone:** 10 minutes at –20°C. Air dry. Proceed with staining procedure immediately.
   - **1c. Methanol:** 10 minutes at –20°C. Proceed with staining procedure immediately.
   - **1d. 3% Formaldehyde:** 15 minutes at room temperature. Proceed with staining procedure immediately.
   - **1e. 3% Formaldehyde/methanol:** 15 minutes at room temperature, followed by 5 minutes in methanol at –20°C **(do not rinse in between)**. Proceed with staining procedure immediately.

D Staining

1. Wash sections in wash buffer twice for 5 minutes.
2. Incubate for 10 minutes in 3% H_2O_2 diluted in methanol at room temperature.
3. Wash sections in wash buffer twice for 5 minutes.
4. Block each section with blocking solution for one hour at room temperature.
5. Remove blocking solution and add 100-400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution). Incubate overnight at 4°C.
   **Refer to product datasheet to determine the recommended dilution.*
6. Remove antibody solution and wash sections three times with wash buffer for 5 minutes each.
7. Add 100-400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
8. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
9. Remove secondary antibody solution and wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 µl ABC reagent to each section and incubate for 30 min. at room temperature.
11. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
12. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
13. As soon as the sections develop, immerse slides in dH_2O.
14. If desired, counterstain sections in Hematoxylin per manufacturer's instructions.
15. Wash sections in dH_2O two times for 5 minutes each.
16. **Dehydrate sections:**
    - **16a.** Incubate sections in 95% ethanol two times for 10 seconds each.
    - **16b.** Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    - **16c.** Repeat in xylene, incubating sections two times for 10 seconds each.
17. Mount coverslips.