#12692

SignalStain® Apoptosis (Cleaved Caspase-3) IHC Detection Kit

☑ 1 Kit
(120 slides)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 11/04/13

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

The components in **13217** ship together at 4°C. Upon receipt and for long-term storage, please refer to the individual kit component storage temperatures.

Components Ship As: 13217	Item #	Kit Quantity	Storage Temp
Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb	9579	1 x 100 µl	–20°C
Rabbit (DA1E) mAb IgG XP® SignalStain® Isotype Control	12960	1 x 100 µl	–20°C
Components Ship As: 13217	**Item #**	**Kit Quantity**	**Storage Temp**
Tris Buffered Saline with Tween® 20 (TBST-10X)	9997	1 x 2.4 ml	Room Temp.
Components Ship As: 13217	**Item #**	**Kit Quantity**	**Storage Temp**
SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)	8114	1 x 12 ml	4°C
SignalStain® DAB Diluent	11724	1 x 12 ml	4°C
SignalStain® DAB Chromogen Concentrate	11725	1 x 360 µl	4°C
Components Ship As: 8112S	**Item #**	**Kit Quantity**	**Storage Temp**
SignalStain® Antibody Diluent	8112	1 x 25 ml	4°C
Components Ship As: 5425P	**Item #**	**Kit Quantity**	**Storage Temp**
Normal Goat Serum	5425	1 X 2.4 ml	–20°C

Description: SignalStain® Apoptosis (Cleaved Caspase-3) IHC Detection Kit allows the detection of activated caspase-3 in formalin-fixed paraffin-embedded human and mouse tissue samples. Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb is detected by the polymer based, HRP-conjugated SignalStain® Boost IHC Detection Reagent in combination with SignalStain® DAB Diluent and Chromogen Concentrate. Also included is a concentration-matched rabbit monoclonal IgG control to verify the specificity of staining.

This combination of reagents provides a sensitive and specific means of detecting apoptotic events in tissue samples.

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. Cleavage of caspase-3 requires the aspartic acid residue at the P1 position (2).

Specificity/Sensitivity: SignalStain® Apoptosis (Cleaved Caspase-3) IHC Detection Kit detects endogenous levels of the activated caspase-3 large fragment (17/19 kDa) resulting from cleavage adjacent to Asp175. This antibody does not recognize full-length caspase-3 or other cleaved caspases. This kit was developed for and is recommended for immunohistochemistry only.

Source/Purification: Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp175 of human caspase-3 protein. Rabbit (DA1E) mAb IgG XP® SignalStain® Isotype Control is not directed against any known antigen. It functions as an isotype control for rabbit IgG monoclonal antibodies.

Background References:

(1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761-30764.

(2) Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.

Storage:
Tris Buffered Saline with Tween® 20 (TBST-10X) should be stored at room temperature.
SignalStain® Boost IHC Detection Reagent (HRP, Rabbit), SignalStain® DAB Diluent, SignalStain® Antibody Diluent and SignalStain® DAB Chromogen Concentrate should be stored at 4°C.
Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb, Rabbit (DA1E) mAb IgG XP® SignalStain® Isotype Control, and Normal Goat Serum should be stored at -20°C.

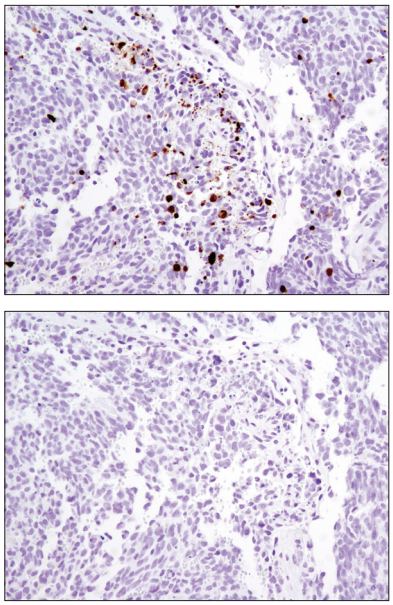
This kit is stable for 12 months.

Reagents Not Supplied:

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH_2O)
4. Tris Buffered Saline with Tween® 20 (TBST-10X) #9997 (sufficient for washes)
5. 10 mM Sodium Citrate Buffer, pH 6.0
6. Hydrogen Peroxide 3% solution
7. Hematoxylin (optional)

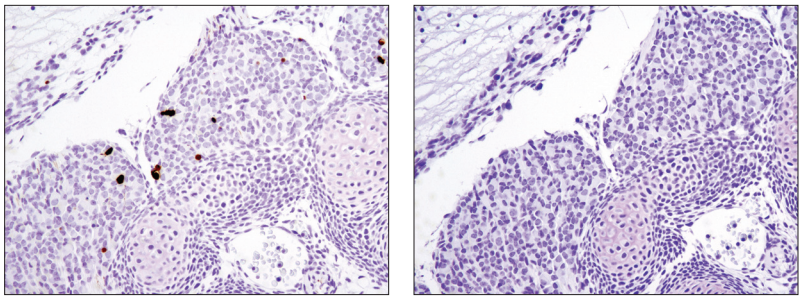
Recommended Antibody Dilutions:

Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb	1:500
Rabbit (DA1E) mAb IgG XP® SignalStain Isotype Control	1:500



Immunohistochemical analysis of paraffin-embedded Kelly xenograft using Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb #9579 (upper) or Rabbit (DA1E) mAb IgG XP® SignalStain® Isotype Control #12960 (lower).

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 E14 mouse embryo using Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb #9579 (left) or Rabbit (DA1E) mAb IgG XP® SignalStain® Isotype Control #12960 (right).

#12692

SignalStain® Apoptosis (Cleaved Caspase-3) IHC Detection Kit Protocol

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:**
   NOTE: Additional 10X TBST will be required for washes.
   a. **Tris Buffered Saline with Tween® 20 (TBST-10X) #9997:**
   To prepare wash buffer add 100 ml of Tris Buffered Saline with Tween® 20 (TBST-10X) #9997 to 900 ml of dH_2O. OR
   b. **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.
   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.
6. **Antibody Diluent:** SignalStain® Antibody Diluent #8112
7. **Antigen Unmasking:** 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.
8. **3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H_2O_2 to 90 ml dH_2O.
9. **Blocking Solution:** 1X TBST/5% normal goat serum:
   Add 100 µl 10X TBST (#9997) and 50 µl normal goat serum (#5425) to 850 µl dH_2O.
10. **Detection System:** SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) (#8114).
11. **Substrate:** SignalStain® DAB Substrate Kit (#8059), which includes SignalStain® DAB Diluent (#11724) and SignalStain® DAB Chromogen Concentrate (#11725).

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 min each.
   b. Incubate sections in two washes of 100% ethanol for 10 min each.
   c. Incubate sections in two washes of 95% ethanol for 10 min each.
2. Wash sections twice in dH_2O for 5 min each.

C Antigen Unmasking

1. Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0, then maintain at a sub-boiling temperature for 10 min. Cool slides on bench top for 30 min.

D Staining

1. Wash sections in dH_2O three times for 5 min each.
2. Incubate sections in 3% hydrogen peroxide for 10 min.
3. Wash sections in dH_2O twice for 5 min each.
4. Wash section in wash buffer for 5 min.
5. Block each section with 100-200 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100–200 µl primary antibody diluted at 1:500 in SignalStain® Antibody Diluent #8112 to each section. Incubate overnight at 4°C.
   a. Rabbit (DA1E) mAb XP® SignalStain® Isotype Control is used as a negative control. Dilute at 1:500 in SignalStain® Antibody Diluent and apply 100-200 µl to each section.
7. Equilibrate SignalStain® Boost Detection Reagent to room temperature.
8. Remove antibody solution and wash sections in wash buffer three times for 5 min each.
9. Cover section with 1–3 drops SignalStain® Boost Detection Reagent as needed. Incubate in a humidified chamber for 30 min at room temperature.
10. Wash sections three times with wash buffer for 5 min each.
11. Add 30 µl SignalStain® DAB Chromogen Concentrate (#11725) to 1 ml SignalStain® DAB Diluent (#11724) and mix well before use.
12. Apply 100–400 µl SignalStain® DAB to each section and monitor closely. 1–10 minutes generally provides an acceptable staining intensity.
13. 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 min each.
16. **Dehydrate sections:**
    a. Incubate sections in 95% ethanol two times for 10 sec each.
    b. Repeat in 100% ethanol, incubating sections two times for 10 sec each.
    c. Repeat in xylene, incubating sections two times for 10 sec each.
17. Mount coverslips.