

Store at
4°C

#13079

Immunohistochemistry Application Solutions Kit (Rabbit)

www.cellsignal.com

1 Kit (120 slides)

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

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New 06/15

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

Applications
IHC-P

Species Cross-Reactivity
All

Products Included	Item #	Kit Quantity	Storage Temp
SignalStain® Antibody Diluent	8112	25 ml	4°C
SignalStain® Boost IHC Detection Reagent (HRP, Rabbit)	8114	12 ml	4°C
SignalStain® DAB Diluent	11724	12 ml	4°C
SignalStain® DAB Chromogen Concentrate	11725	360 µl	4°C
Animal-Free Blocking Solution (5X)	15019	4 ml	4°C

Description: The Immunohistochemistry Application Solutions Kit (IHC-P) is designed to conveniently provide supporting reagents needed for immunohistochemistry staining in paraffin-embedded tissue samples or cell pellets (IHC-P). The reagents in this kit are thoroughly validated using our IHC-recommended **rabbit polyclonal and monoclonal antibodies** and will perform optimally with the CST immunohistochemistry staining protocol, ensuring accurate and reproducible results. This kit includes sufficient reagents for 120 slides based on a 100 µl assay volume. All reagents in this kit are available individually.

IMPORTANT: Please refer to the primary antibody data sheet to determine if the antibody is approved for use on paraffin-embedded samples (IHC-P) and for information regarding the appropriate antibody dilution, diluent, and antigen unmasking procedure.

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.

Reagents not supplied:

1. Xylene
2. Ethanol anhydrous denatured, histological grade (100% and 95%)
3. Deionized Water (dH₂O)
4. Antigen unmasking reagent
5. 3% Hydrogen Peroxide
6. Primary antibody
7. Tris-Buffered Saline with Tween® 20 (TBST-10X, #9997)
8. Hematoxylin #14166 (optional)
9. SignalStain® Mounting Medium #14177

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

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry 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.

Immunohistochemistry Application Solutions Kit Protocol

NOTE: Please refer to primary antibody datasheet or product web page for recommended primary antibody dilution, antibody diluent, and antigen unmasking procedure.

A Solutions and Reagents

SUPPLIED REAGENTS

- Antibody Diluent: SignalStain® Antibody Diluent: (#8112)**
- IHC Detection System: SignalStain® Boost IHC Detection Reagent (HRP, Rabbit): (#8114)**
- SignalStain® DAB Substrate Kit: (#8059)**
 - SignalStain® DAB Diluent (#11724)
 - SignalStain® DAB Chromogen Concentrate (#11725)
- Animal-Free Blocking Solution (5X): (#15019)**
1.0 ml of 1X Blocking Solution, add 200 µl of Animal-free blocking solution and 800 µl of dH₂O just before use. Always prepare fresh 1X solutions daily.

ADDITIONAL REAGENTS (NOT SUPPLIED)

- Xylene**
- Ethanol**, anhydrous denatured, histological grade (100% and 95%)
- Deionized water** (dH₂O)
- Antigen Unmasking:**
NOTE: Consult product datasheet for specific unmasking solution recommendation.
 - Citrate:** 10 mM Sodium Citrate Buffer, pH 6.0: To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 1 L dH₂O. Adjust pH to 6.0.
 - EDTA:** 1 mM EDTA, pH 8.0: To prepare 1 L, add 0.372 g EDTA (C₁₀H₁₄N₂O₈Na₂•2H₂O) to 1 L dH₂O. Adjust pH to 8.0.
 - TE:** 10 mM Tris/1 mM EDTA, pH 9.0: To prepare 1 L, add 1.21 g Trizma® base (C₄H₁₁NO₃) and 0.372 g EDTA (C₁₀H₁₄N₂O₈Na₂) to 950 ml dH₂O. Adjust pH to 9.0 and adjust final volume to 1 L with dH₂O.
 - Pepsin:** 1 mg/ml in Tris-HCl, pH 2.0.
- 3% Hydrogen Peroxide:** To prepare 100 ml, add 10 ml 30% H₂O₂ to 90 ml dH₂O.
- Primary antibody**
- Wash Buffer:** To prepare 1 L 1X TBST wash buffer: add 100 ml of 10X Tris Buffered Saline with Tween® 20 (TBST) (#9997) to 900 ml of dH₂O, mix.
- Hematoxylin #14166** (optional)
- SignalStain® Mounting Medium #14177**

B Deparaffinization/Rehydration

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

- Deparaffinize/hydrate sections:**
 - Incubate sections in three washes of xylene for 5 min each.
 - Incubate sections in two washes of 100% ethanol for 10 min each.
 - Incubate sections in two washes of 95% ethanol for 10 min each.
- Wash sections twice in dH₂O for 5 min each.

C Antigen Unmasking

NOTE: Consult product datasheet for specific unmasking solution recommendation.

- For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer, pH 6.0 and maintain at a sub-boiling temperature for 10 min. Cool slides on bench top for 30 min.
- For EDTA:** Bring slides to a boil in 1 mM EDTA, pH 8.0 and follow with 15 min at a sub-boiling temperature. No cooling is necessary.
- For TE:** Bring slides to a boil in 10 mM Tris/1 mM EDTA, pH 9.0 and maintain at a sub-boiling temperature for 18 min. Cool at room temperature for 30 min.
- For Pepsin:** Digest for 10 min at 37°C.

D Staining

NOTE: Consult product datasheet for specific dilution recommendations for each primary antibody.

- Wash sections in dH₂O three times for 5 min each.
- Incubate sections in 3% hydrogen peroxide for 10 min.
- Wash sections in dH₂O twice for 5 min each.
- Wash section in wash buffer for 5 min.
- Block each section with 100–150 µl blocking solution for 1 hr at room temperature.
- Remove blocking solution and add 100–200 µl primary antibody diluted in SignalStain® Antibody Diluent (#8112) to each section. Incubate overnight at 4°C.
- Equilibrate SignalStain® Boost Detection Reagent (#8114) to room temperature.
- Remove antibody solution and wash sections in wash buffer three times for 5 min each.
- Cover section with 1–3 drops SignalStain® Boost Detection Reagent as needed. Incubate in a humidified chamber for 30 min at room temperature.
- Wash sections three times with wash buffer for 5 min each.
- Add 1 drop (30 µl) SignalStain® DAB Chromogen Concentrate to 1 ml SignalStain® DAB Diluent and mix well before use.
- Apply 100–400 µl SignalStain® DAB to each section and monitor closely. 1–10 min generally provides acceptable staining intensity.
- Immerse slides in dH₂O.
- If desired, counterstain sections in Hematoxylin #14166.
- Wash sections in dH₂O two times for 5 min each.
- Dehydrate sections:
 - Incubate sections in 95% ethanol two times for 10 sec each.
 - Repeat in 100% ethanol, incubating sections two times for 10 sec each.
 - Repeat in xylene, incubating sections two times for 10 sec each.
- Mount coverslips using SignalStain® Mounting Medium #14177.
 - Apply a small amount of SignalStain® Mounting Medium #14177 either on or near the section using a pipette. When the coverslip is applied, the medium will spread evenly across the coverslipped area.
 - Allow the preparation to dry completely. Drying may take several hours when left on the lab bench, however this process can be accelerated by incubating the slides for 15–30 minutes at 60°C to 75°C. Please ensure the slides have cooled before storage.

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