Applications	Reactivity	Sensitivity	MW (kDa)	Source
W IF-IC	H M R	Endogenous	69, 78	Rabbit

Applications Key: W=Western Blotting IF-IC=Immunofluorescence (Immunocytochemistry)

Reactivity Key: H=Human M=Mouse R=Rat

Species cross-reactivity is determined by western blot. Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Protocols

Immunofluorescence

Immunofluorescence General Protocol

IMPORTANT: Please refer to the APPLICATIONS section on the front page of product datasheet to determine if this product is validated and approved for use on cultured cell lines (IF-IC), paraffin-embedded samples (IF-P), or frozen tissue sections (IF-F). Please see product datasheet for appropriate antibody dilution and unmasking solution.

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **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 8.0.
2. **Formaldehyde:** 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4 ℃ in dark, dilute in PBS for use.
3. **Blocking Buffer:** (1X PBS / 5% normal goat serum (#5425) / 0.3% Triton™ X-100): To prepare 25 ml, add 2.5 ml 10X PBS, 1.25 ml normal serum from the same species as the secondary antibody (e.g., normal goat serum, normal donkey serum) and 21.25 ml dH_2O and mix well. While stirring, add 75 µl Triton™ X-100.
4. **Antibody Dilution Buffer:** (1X PBS / 1% BSA / 0.3% Triton™ X-100): To prepare 40 ml, add 4 ml 10X PBS and 120 µl Triton™ X-100 to 0.4 g BSA. Bring to final volume of 40 ml with dH_2O and mix well.
5. **Fluorochrome-conjugated secondary antibody NOTE:** When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.
6. **Prolong® Gold Anti-Fade Reagent** (#9071), with DAPI (#8961).

Reagents specific to IF-P application:

1. **Xylene**
2. **Ethanol**, anhydrous denatured, histological grade, 100% and 95%.
3. **Antigen Unmasking:**

a. **For Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7•2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.

b. **For EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA ($C_{10}H_{14}N_2O_8Na_2•2H_2O$) to 1 L dH_2O. Adjust pH to 8.0.

B. Specimen Preparation

I. Cultured Cell Lines (IF-IC)

NOTE: Cells should be grown, treated, fixed and stained directly in multi-well plates, chamber slides or on coverslips.

1. Aspirate liquid, then cover cells to a depth of 2–3 mm with 4% formaldehyde in PBS. **NOTE:** Formaldehyde is toxic, use only in fume hood.
2. Allow cells to fix for 15 min at room temperature.
3. Aspirate fixative, rinse three times in PBS for 5 min each.
4. Proceed with Immunostaining (Section C).

II. Paraffin Sections (IF-P)

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

1. **Deparaffinization/Rehydration:**

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.

d. Rinse sections twice in dH_2O for 5 min each.

2. **Antigen Unmasking:**

NOTE: Consult product datasheet for specific recommendation for the unmasking solution.

2.

a. **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 min. Cool slides on bench top for 30 min.

b. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 min at a sub-boiling temperature. No cooling is necessary.

3. Proceed with Immunostaining (Section C).

III. Frozen/Cryostat Sections (IF-F)

1. For fixed frozen tissue proceed with Immunostaining (Section C).
2. For fresh, unfixed frozen tissue, please fix immediately, as follows:

a. Cover sections with 4% formaldehyde in PBS.

b. Allow sections to fix for 15 min at room temperature.

c. Rinse slides three times in PBS for 5 min each.

d. Proceed with Immunostaining (Section C).

C. Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in Blocking Buffer for 60 min.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary antibody.

4. Incubate overnight at 4 ℃.
5. Rinse three times in PBS for 5 min each.

   NOTE: If using primary antibodies directly conjugated with Alexa Fluor® fluorochromes, then skip to (Section C, Step 8).
6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1–2 hr at room temperature in dark.
7. Rinse in PBS (Section C, Step 5).
8. Coverslip slides with Prolong® Gold Anti-Fade Reagent (#9071), with DAPI (#8961).
9. For best results, allow mountant to cure overnight at room temperature. For long-term storage, store slides flat at 4 ℃ protected from light.

Western Blotting

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4 ℃ with gentle shaking, overnight.

Products available from Cell Signaling Technology are linked by their respective catalog numbers.

A. Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. **1X Phosphate Buffered Saline (PBS).**
2. **1X SDS Sample Buffer:** (#7722, #7723) 62.5 mM Tris-HCl (pH 6.8 at 25 ℃), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red.
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5).
4. **10X Tris Buffered Saline (TBS):** (#9997) To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. **Nonfat Dry Milk:** (#9999) (weight to volume [w/v]).
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T).
8. **Bovine Serum Albumin (BSA):** (#9998).
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System:** (#7071 anti-rabbit) or (#7072 anti-mouse) Includes biotinylated protein ladder, secondary (#7074 anti-rabbit) or (#7076 anti-mouse) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720).
12. **Biotinylated Protein Ladder Detection Pack:** (#7727).

13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B. Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds for complete cell lysis and to shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100 ℃ for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.
8. Electrotransfer to nitrocellulose or PVDF membrane.

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4 ℃.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.

I. For Unconjugated Primary Antibodies

1. Incubate membrane with **appropriate** HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
2. Wash three times for 5 minutes each with 15 ml of TBS/T.

II. For HRP Conjugated Primary Antibodies

Skip to Detection of Proteins (Step D).

III. For Biotinylated Primary Antibodies

1. Incubate membrane with HRP-Streptavidin (at the appropriate dilution) in milk for one hour with gentle agitation at room temperature.
2. Wash three times for 5 minutes each with 15 ml of TBS/T.

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature. **NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

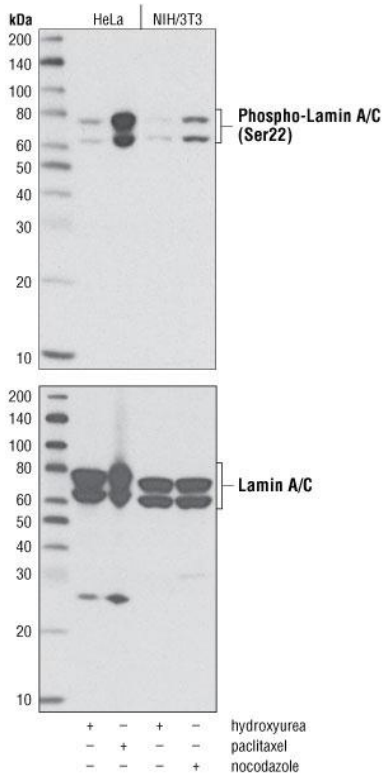
Specificity / Sensitivity

Phospho-Lamin A/C (Ser22) Antibody detects endogenous levels of lamin A/C only when phosphorylated at Ser22.

Source / Purification

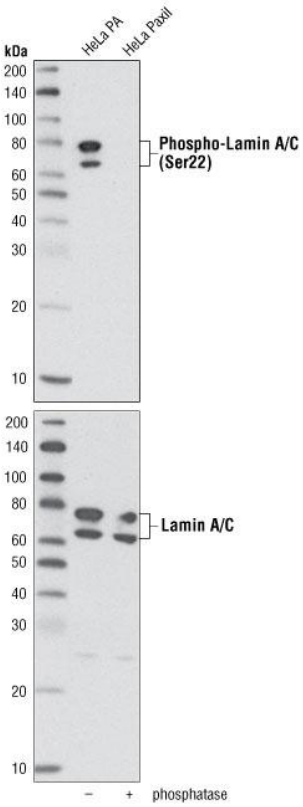
Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser22 of human lamin A/C protein.

Western Blotting



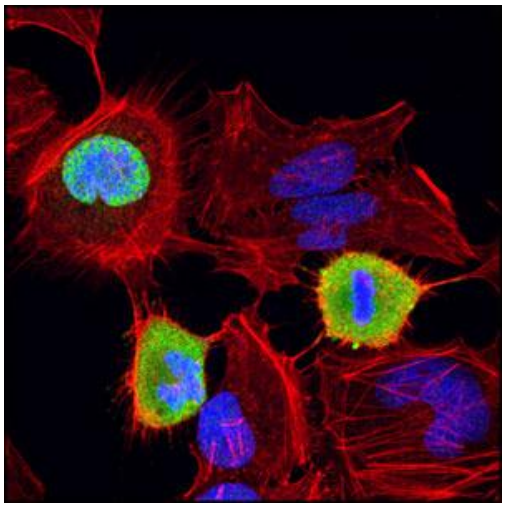
Western blot analysis of extracts from HeLa and NIH/3T3 cells, hydroxyurea-treated (4 mM, 20 hours) to induce G1/S phase or paclitaxel-treated (100 nM, 20 hours) or nocodazole-treated (100 ng/ml, 20 hours) to induce G2/M phase, using Phospho-Lamin A/C (Ser22) Antibody (upper) or Lamin A/C Antibody #2032 (lower) as a loading control.

Western Blotting



Western blot analysis of extract from G2/M phase HeLa cells untreated or treated with phosphatase, using Phospho-Lamin A/C (Ser22) Antibody (upper) or Lamin A/C Antibody (lower) as loading control.

IF-IC



Confocal immunofluorescent analysis of HeLa cells using Phospho-Lamin A/C (Ser22) Antibody (green). Actin filaments have been labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5™ (fluorescent DNA dye).