#3875 Store at -20°C

Aurora Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

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Support ■ 877-678-TECH (8324)
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rev. 03/08/16

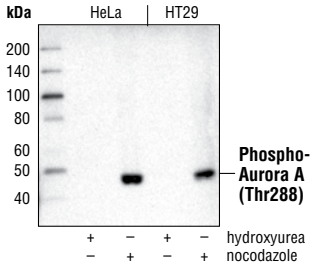
For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Aurora A (Thr288) (C39D8) Rabbit mAb	3079	20 µl	48 kDa	Rabbit IgG
Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) (D13A11) XP® Rabbit mAb	2914	20 µl	35, 40, 48 kDa	Rabbit IgG
Aurora A (D3E4Q) Rabbit mAb	14475	20 µl	48 kDa	Rabbit IgG
Aurora B/AIM1 Antibody	3094	20 µl	40 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Description: The Aurora Antibody Sampler Kit provides an economical means to investigate the G2/M phase of the cell cycle. The kit contains enough primary and secondary antibodies to perform two Western mini-blots with each antibody.

Background: Aurora kinases belong to a highly conserved family of mitotic serine/threonine kinases with three members identified among mammals: Aurora A, Aurora B and Aurora C (1,2). Studies on the temporal expression pattern and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Their functional influences span from G2 to cytokinesis and may be involved in key cell cycle events such as centrosome duplication, chromosome bi-orientation and segregation, cleavage furrow positioning and ingression (3). Aurora A is detected at the centrosomes, along mitotic spindle microtubules and in the cytoplasm of mitotically proliferating cells. Aurora A protein levels are low during G1 and S phases and peak during the G2/M phase of the cell cycle. Phosphorylation of Aurora A at Thr288 in its catalytic domain increases kinase activity. Aurora A is involved in centrosome separation, maturation and spindle assembly and stability. Expression of Aurora B protein also peaks during the G2/M phase of the cell cycle, while kinase activity peaks at the transition from metaphase to the end of mitosis. Aurora B associates with chromosomes during prophase prior to relocalizing to the spindle at anaphase. Aurora B regulates chromosome segregation through the control of microtubule-kinetochore attachment and cytokinesis. Expression of both Aurora A and Aurora B during the G2/M phase transition is tightly coordinated with histone H3 phosphorylation (4,5), while overexpression of both kinases is seen in a variety of human cancers (2,4). Aurora C localizes to the centrosome from anaphase to cytokinesis and both mRNA and protein levels peak during G2/M phase. Although typical Aurora C expression is limited to the testis, overexpression of Aurora C is detected in various cancer cell lines (6).



Western blot analysis of extracts from HeLa and HT29 cells, hydroxyurea or nocodazole treated, ***Phospho-Aurora A (Thr288) (C39D8) Rabbit mAb #3079.***

Specificity/Sensitivity: Each antibody in the Aurora Antibody Sampler Kit detects endogenous levels of its respective target protein and does not cross-react with other family members.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide and are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with recombinant human proteins or synthetic peptides.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibodies.*

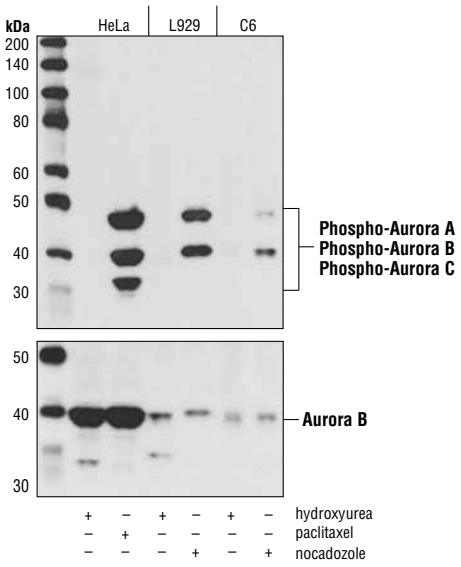
Recommended Antibody Dilutions:
Western blotting 1:1000

Note: Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) (D13A11) Rabbit mAb 1:2000

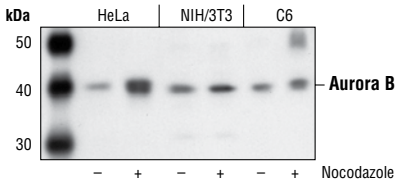
Please visit www.cellsignal.com for a complete listing of recommended companion products.

U.S. Patent No. 5,675,063

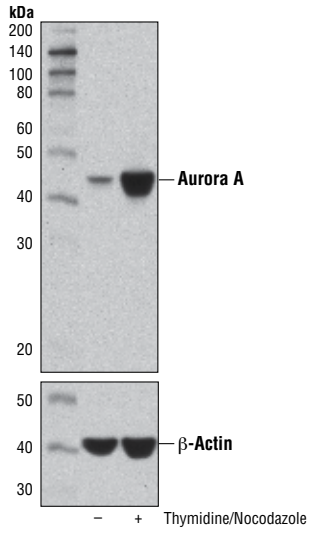
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from HeLa, L929 and C6 cells, treated with 4 mM hydroxyurea for 20 hours or treated with 100 nM paclitaxel or 100 ng/ml nocodazole for 20 hours, using ***Phospho-Aurora A (Thr288)/Aurora B (Thr232)/Aurora C (Thr198) D11A13 XP® Rabbit mAb #2914*** *(upper) or* ***Aurora B/AIM1 Antibody #3094*** *(lower).*



Western blot analysis of extracts from HeLa, NIH/3T3 and C6 cells, untreated or nocodazole-treated (50 ng/ml, 24 hrs), using ***Aurora B/AIM1 Antibody #3094.***



Western blot analysis of extracts from HT-29 cells, untreated (-) or synchronized in mitosis by treatment with thymidine (2 mM, 17 hr; +) and Nocodazole #2190 (100 ng/ml, 24 hr; +), using ***Aurora A (D3E4Q) Rabbit mAb #14475*** *(upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).*

Background References:

(1) Warner, S.L. et al. (2003) *Mol. Cancer Ther.* 2, 589–595.

(2) Katayama , H. et al. (2003) *Cancer Metastasis Rev.* 22, 451–464.

(3) Andrews, P.D. et al. (2003) *Curr. Opin. Cell Biol.* 15, 672–683.

(4) Pascreau, G. et al. (2003) *Prog. Cell Cycle Res.* 5, 369–374.

(5) Crosio, C. et al. (2002) *Mol. Cell. Biol.* 22, 874–885.

(6) Kimura, M. et al. (1999) *J. Biol. Chem.* 274, 7334–7340.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.