Store at -80°C

#7513

HTScan® Aurora B Kinase Assay Kit

☑100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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rev. 07/14/08

Products Included	Products #	Kit Quantity
Phospho-PLK (Ser137) Antibody	5070	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
PLK (Ser137) Biotinylated Peptide	1300	1.25 ml
Aurora B Kinase (recombinant, human)	7394	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human Aurora B kinase. It includes active Aurora B kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: RRS*LL

Molecular Weights: Biotin-PLK (Ser137) : 1,945 Daltons. GST-Aurora B Kinase: 66 kDa.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human Aurora B (Met1-Ala344) (GenBank Accession No. NM_004217) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

Quality Control: The substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-PLK (Ser137) Antibody #5070 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified Aurora B kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain. The specific activity of the Aurora B kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify Aurora B activity using the Aurora B substrate peptide provided in this kit. Aurora B sensitivity to the inhibitor staurosporine was measured using the Aurora B substrate peptide provided in this kit [Fig.5].

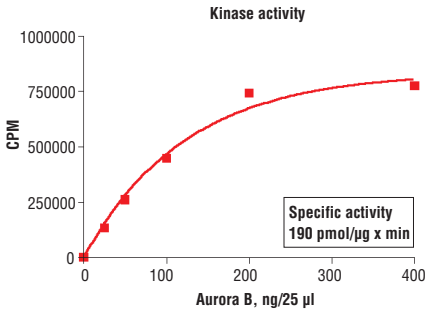

Figure 1. Aurora B kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: MBP 200 ng/µL, and recombinant Aurora B: variable.

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO.

Enzyme is supplied in 50 mM Tris-HCl, pH7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

Aurora B Kinase #7394

Phospho-PLK (Ser137) Antibody #5070

PLK (Ser137) Biotinylated Peptide #1300

Staurosporine #9953

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

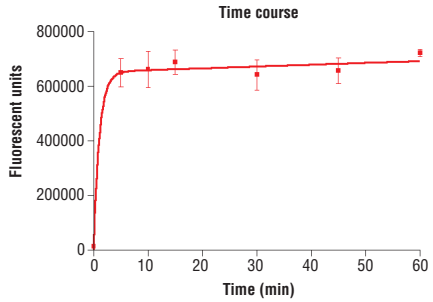

Figure 2. Time course of Aurora B kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of Aurora B substrate peptide #1300 by Aurora B kinase. In a 50 µl reaction, 50 ng Aurora B and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

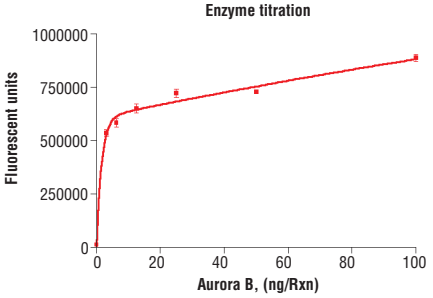

Figure 3. Dose dependence curve of Aurora B kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide #1300 by Aurora B kinase. In a 50 µl reaction, increasing amounts of Aurora B and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

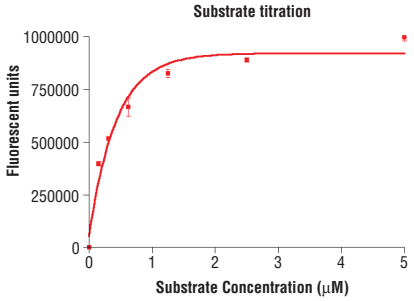

Figure 4. Peptide concentration dependence of Aurora B kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide #1300 by Aurora B kinase. In a 50 µl reaction, 50 ng of Aurora B and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

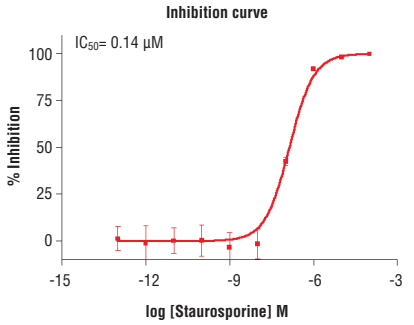

Figure 5. Staurosporine inhibition of Aurora B kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of Aurora B substrate peptide #1300 by Aurora B kinase. In a 50 µl reaction, 50 ng Aurora B, 1.5 µM substrate peptide, 50 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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 pattern of expression and subcellular localization of Aurora kinases in mitotic cells suggest an association with mitotic structure. Their functional influences span from G2 through to cytokinesis and may be involved in key cell cycle events such as centrosome duplication, chromosome biorientation and segregation, cleavage furrow positioning and ingression (3). Aurora A is detected in mitotically proliferating cells at the centrosomes, along microtubules of the mitotic spindle and in cytoplasm. Its protein levels are low during G1 and S phases and peak during the G2/M phase of the cell cycle. Phosphorylation of Thr288 in its catalytic domain increases kinase activity. Aurora A is involved in centrosome separation, maturation and spindle assembly and stability. Overexpression of Aurora A has been detected in human breast, bladder, colon, ovarian and pancreatic cancers (2,4). The expression of Aurora B also peaks during the G2/M phase of the cell cycle and the kinase activity peaks at the transition from metaphase to the end of mitosis. Aurora B associates with chromosomes during prophase and then relocalizes to the spindle at anaphase. Aurora B regulates chromosome segregation through the control of microtubule-kinetochore attachment and cytokinesis. Aurora B overexpression is also detected in a variety of human cancers (2,4). The expression of both Aurora A and Aurora B is tightly coordinated with histone H3 phosphorylation during the G2/M phase transition (4,5). Aurora C localizes on the centrosome from anaphase to cytokinesis and expression of both mRNA and protein levels peaks during G2/M phase. Although the tissue distribution of Aurora C shows that its expression is limited to the testis, overexpression of Aurora C is detected in various cancer cell lines (6).

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.

Protocol for HTScan® Aurora B Kinase Assay Kit

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [1 ml 10X Kinase Buffer 250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Transfer 0.6 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 8 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng Aurora B Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody, Phospho-PLK (Ser137) Antibody, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 405 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

***NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com