Rabbit (polyclonal) Anti-Histone H3 [pS^{10}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	441190G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Histone H3. The final product is generated by affinity chromatography using a Histone H3-derived peptide that is phosphorylated at serine 10.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Histone H3 that contains serine 10.
Target Summary:	Histone H3 (17 kDa) is a member of Histone family of proteins (H2A, H2B, H3, and H4) which are the building blocks of chromatin. Histones undergo several post-translational modifications, including acetylation, phosphorylation, methylation and ubiquitination. Histone H3 plays an important role in regulating chromatin organization and hetero-chromatization which is critical for controlling gene expression and silencing, and represent a potential target for cancer chemotherapy. Histone H3 is phosphorylated on serine 10 by several kinases included in the Aurora kinase family. Phosphorylation of Histone H3 is implicated in transcriptional activation and chromosome condensation. Mitotic phosphorylation of Histone H3 at serine 10 is critical for proper chromosome condensation and segregation. Phosphorylation of Histone H3 at Ser10 is also essential for EGF-induced neoplastic cell transformation.
Reactivity:	Human Histone H3. Mouse and Rat (100% homologous) Histone H3 have not been tested, but are expected to react.
Applications:	The antibody has been used in Western blotting. Other applications may work but have not been tested.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The exact concentration is not determined for each lot; however, the typical range is 0.1-1.0 mg/mL. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2 to 8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Controls Used:	Sulfuric acid extracted thymidine–treated NIH3T3 cells.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

PI441190G (Rev 01/09) DCC-08-1089

Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Related Products:

Antibodies:

Aurora A Kinase [pT^{288}], Cat. # 441210G
TTK [pTpSpS$^{360/362/363}$], Cat. # 441330G
TTK [pTpS$^{33/37}$], Cat. # 441325G
c-Jun [pS^{73}], Cat. # 44292G
c-Fos [pT^{232}], Cat. # 44280G
c-Fos [pT^{325}], Cat. # 44281G
ATF2 [pT^{71}], Cat. # 44295G
ATF2 [pTpT$^{69/71}$], Cat. # 44294G
FOXO1 [pT^{24}]/FOXO3 [pT^{38}], Cat. # 441240G
FOXO3 [pS^{207}]/FOXO1 [pS^{212}], Cat. # 441230G
FOXO1 [pS^{246}], Cat. # 441245G

References:

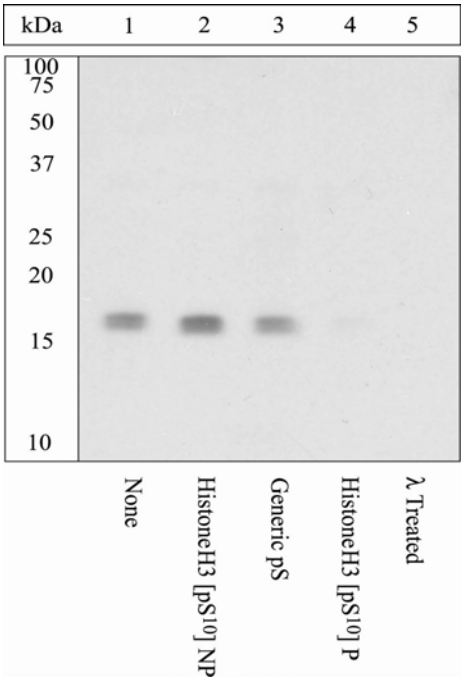
Johansen, K.M. and J. Johansen. (2006) Regulation of chromatin structure by Histone H3 S10 phosphorylation. Chromosome Res. 14(4):393-404 (Review).

Sng, J.C., et al. (2006) Histone modifications in kainate-induced status epilepticus. Eur. J. Neurosci. 23(5):1269-1282.

Huang, X., et al. (2006) Sequential phosphorylation of Ser-10 on Histone H3 and ser-139 on Histone H2AX and ATM activation during premature chromosome condensation: relationship to cell-cycle phase and apoptosis. Cytometry A. 69(4):222-229.

Hirota, T., et al. (2005) Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. Nature 438(7071):1176-1180.

Choi, H.S., et al. (2005) Phosphorylation of Histone H3 at serine 10 is indispensable for neoplastic cell transformation. Cancer Res. 65(13):5818-5827.



Antibody-Peptide Competition and Phosphatase Treatment

Extracts of thymidine–treated NIH3T3 cells (1-5) were resolved on a 10% Tris-glycine gel and transferred to PVDF. The membrane was either left untreated (1-4) or treated with lambda phosphatase (5), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and then incubated with the Histone H3 [pS^{10}] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1), the non-phosphopeptide corresponding to the phosphopeptide immunogen (2), a generic phosphoserine-containing peptide (3), or the phosphopeptide immunogen (4). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that only the phosphopeptide corresponding to Histone H3 [pS^{10}] completely blocks the antibody signal, demonstrating the site-specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

PI441190G (Rev 01/09) DCC-08-1089

Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.
PI441190G (Rev 01/09) DCC-08-1089

Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.