

Certificate of Analysis

Anti-phospho-ATM (Ser1981), clone 10H11.E12

(mouse monoclonal IgG_{1κ})

Catalog # 05-740

Lot # 0701049688

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 1974-1988 (SLAFEEG[pS]QSTTISS) of human ATM. The immunizing sequence has 11/12 identical amino acids in mouse and rat.

Specificity: Recognizes ATM, Mr ~370 kDa. A non-specific protein was also detected, Mr ~ >400 kDa.

Species Cross-reactivity: Human and mouse. Predicted to cross-react with rat based on sequence homology.

Formulation: 200 µg of protein G purified mouse IgG in 200 µL of 0.014M phosphate buffer, pH 7.6, with 0.175M NaCl, 0.07 % Sodium Azide and 30% glycerol. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5 µg/mL of this lot detected phosphorylated ATM in crude lysates from irradiated HeLa cells (Figure A, lanes 1 and 2).

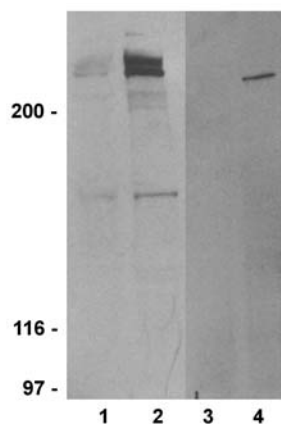


Figure A
Crude cell extracts (lanes 1 and 2) or ATM-containing immune complexes (lanes 3 and 4) from either untreated (lanes 1 and 3) or gamma-irradiated (lanes 2 and 4) HeLa cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-ATM (Ser1981), clone 10H11.E12 (0.5 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated ATM (370 kDa).

Additional Research Applications

Immunoprecipitation: Phosphorylated ATM was immunoprecipitated from irradiated HeLa cells (Figure A, lanes 3 and 4).

Immunocytochemistry: Foci are detected in irradiated human and mouse fibroblasts. Determined by an independent laboratory.

General References:

1. Bartek, J. and J. Lukas (2003). *Nature* **421**: 486-488.
2. Bakkenist, C.J. and M.B. Kastan (2003). *Nature* **421**: 499-506.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (Laemmli sample buffer: 62.5 mM Tris buffer, pH 6.8; 1 mM sodium vanadate; 1 mM sodium fluoride; 2% SDS; 10% glycerol; 5% 2-mercaptoethanol; 0.05% bromophenol blue) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5 µg/mL of anti-phospho-ATM (Ser1981), clone 10H11.E12**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 dilution was used) in TBST-MLK for 1 hour with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in water for 30 minutes.
9. Use detection method of choice (enhanced chemiluminescence was used).