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Human Phospho-ATM (S1981) (S1981) Intracellular ELISA Kit

Catalog number: NR-E10877 (96 wells)

The kit is designed to quantitatively detect the levels of Human Phospho-ATM (S1981) in ***cell lysates.***

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 20 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

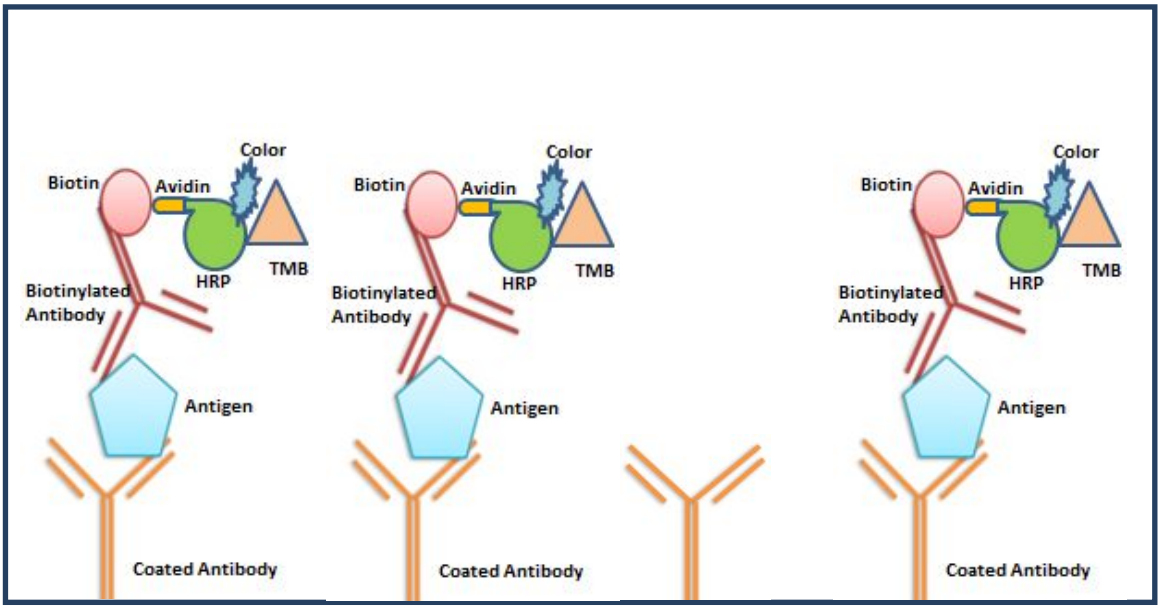
Intended use

The kit is used to quantify the Human Phospho-ATM (S1981) in cell lysates.

Standard range	3.12--200 ng/ml
Sensitivity	3.0 ng/ml
Assay time	4 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Human Phospho-ATM (S1981) ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Human ATM specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated Human Phospho-ATM (S1981) specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the Human Phospho-ATM (S1981) amount of sample captured in plate.



Materials supplied

1.Human Phospho-ATM (S1981) standard:	200 ng/vial ×2.
2. 96-well plate coated with anti-Human Phospho-ATM (S1981) Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Biotinylated Human Phospho-AT M (S1981) Ab:	330 µl, dilution 1:36.
5. Streptavidin-HRP:	1 vial.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. TMB developing agent:	12 ml.
9. Stop Solution:	6 ml.
10. 20 × Wash Buffer:	25 ml.
11. Plate sealer	1.
12. Package insert	1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Cell Lysates - Rinse cells two times with PBS (pH 7.2-7.4), aspirate any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer (1 mM EDTA, 0.5% Triton X-100, 10 g/mL Leupeptin, 10 g/mL Pepstatin, 3 g/mL Aprotinin, 150 mM NaCl, 10 mM NaF, 20 mM , β-glycerophosphate in PBS, pH 7.2-7.4) and allow samples to sit on ice for 15 minutes. Centrifuge samples at 2000 x g for 5 minutes. Collect the supernatants and assay. The user should determine the optimal dilution factor. The remaining samples may be stored at -70°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Standard

- Human Phospho-ATM (S1981): Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (200 ng /vial) are included in each kit. Use one tube for each experiment.
- 200 ng/ml→3.12 ng/ml of Human Phospho-ATM (S1981) standard solutions:
- Add 1 ml of sample diluents into one standard tube with 200 ng Human Phospho-ATM (S1981). Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 200 ng/ml standard solution.
- Label 7 Eppendorf tubes with 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.12 ng/ml, respectively. Make 2-fold serial dilution from 200 ng/ml to 3.12 ng/ml in seven 1.5 ml tubes.
- Make sure each tube has ≥ 300 µl of standard.

Note: The standard solutions are best used within 2 hours.

Biotinylated Human Phospho-ATM (S1981) antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.
- Biotinylated anti-Human Phospho-ATM (S1981) detection antibody should be diluted in 1:36 with Antibody diluent buffer containing 2% heat-inactivated normal mouse serum. Allow the diluted Detection Antibody to sit at least 1-2 hours before use.

Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted in 1:100 with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Human Phospho-ATM (S1981) Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Human Phospho-ATM (S1981) concentration in samples.

1. Add 100 µl of sample or standards per well. Add 100 µl of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 2 hours at room temperature. Note: We recommend that each Human Phospho-ATM (S1981) standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of biotinylated Human Phospho-ATM (S1981) antibody working solution to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 100 µl of TMB developing agent to each well. Cover and incubate for 20-40 minutes at room temperature (Protect from light. Do not over-develop).
8. Add 50µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

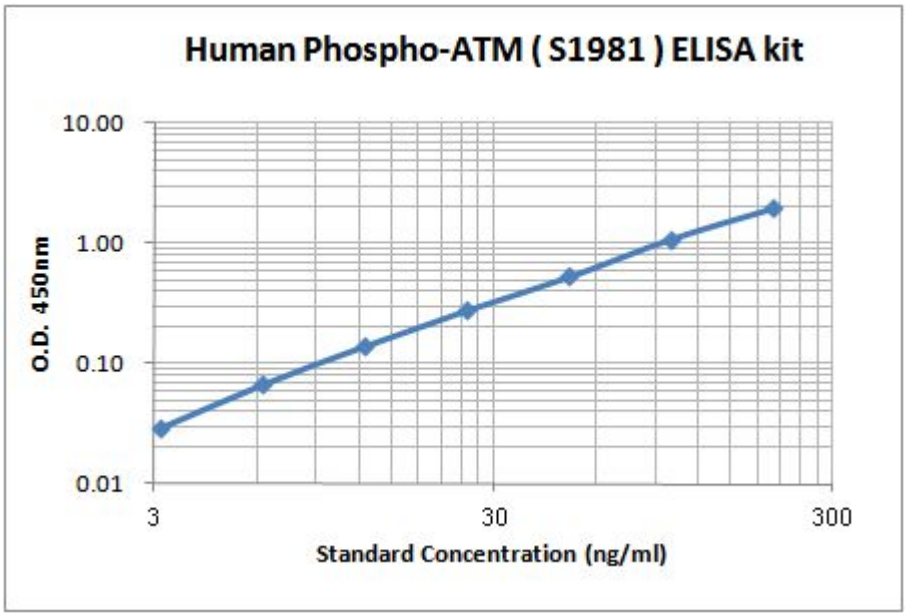
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Phospho-ATM (S1981) concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical data:

This standard curve was generated at Novatein Biolab for demonstration purpose only. A standard curve must be run with each assay.



Background:

Ataxia telangiectasia mutated (ATM) is a serine/threonine protein kinase belonging to the superfamily of Phosphatidylinositol 3-kinase-related kinases (PIKKs).It is a 3056 amino acid (aa) protein characristic of HEAT repeat domain, the FRAP-ATM-TRRAP (FAT) domain, the kinase domain (KD), the PIKK-regulatory domain (PRD) and the FAT-C-terminal (FATC) domain.ATM plays an important role in regulation of DNA damage and repair by phosphorylating several key proteins that initiate activation of the DNA damage checkpoint. ATM is activated through auto- or trans-phosphorylation of Ser-1981 in response to DNA damage, particularly induction of DNA double-strand breaks (DSBs). This phosphorylation provokes dissociation of ATM dimers, which is followed by the release of active ATM monomers.Ataxia telangiectasia (AT) is a rare human disease characterized by cerebellar degeneration, extreme cellular sensitivity to radiation and a predisposition to cancer. All AT patients contain mutations in the ATM gene. ATM defects have been

associated with breast cancer, Mantle cell lymphoma, T-ALL, atypical B cell chronic lymphocytic leukemia, and T-PLL.

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