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Certificate of Analysis

ATM, active
(Recombinant enzyme expressed in mammalian cells)

Item # 14-933, 14-933-K, 14-933M, 14-933S
Parent Lot # WAB0025

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Recombinant, human FLAG-tagged ATM (GenBank NM_000051) full length, expressed in a mammalian cell line.
Purity 68.3% by SDS-PAGE and Coomassie blue staining. MW = 352kDa.

Activity (Parent lot# WAB0025**:** This lot of ATM is active when tested using full length, recombinant p53 (Eurofins cat. 14-952) as the substrate, and meets product specifications.

Formulation: 0.099mg/ml of enzyme in storage buffer.

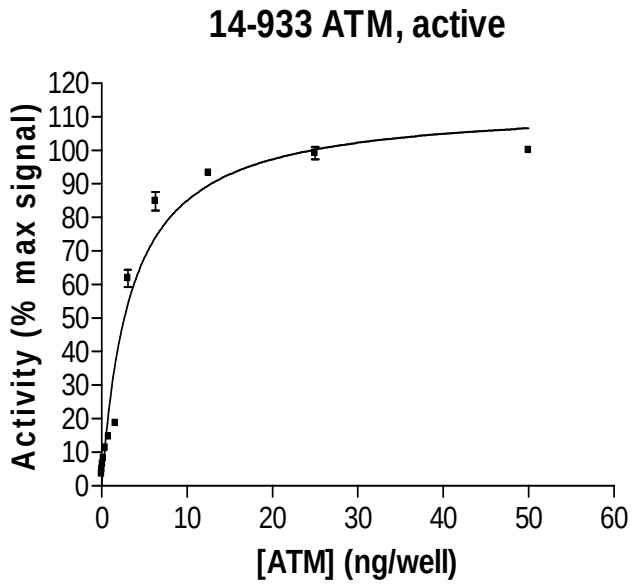
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

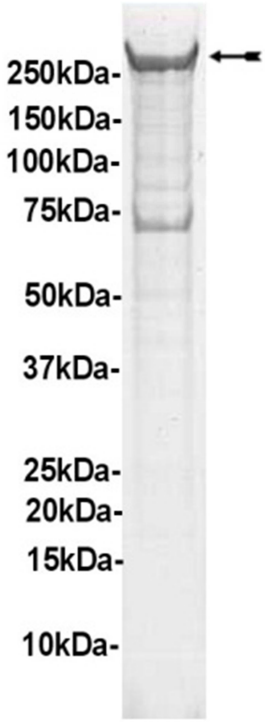
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: 50ng of this enzyme was titrated in an ATM HTRF assay using recombinant, full length human p53 (Eurofins cat. 14-952) as the substrate. The results were normalised against the maximum signal.



MS Tryptic Fingerprint: Confirmed identity as ATM



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active ATM.

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Suggested Kinase Assay Protocol

Reagents:

1. **1 x Reaction Buffer:** 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol.
2. **Dilution Buffer:** 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol, 5mM DTT, 1mg/ml BSA
3. **ATP Solution (4x):** 400µM ATP, 20mM Manganese Chloride, 20mM Magnesium Acetate.
4. **p53 (expressed in *E.coli*) (Eurofins cat. 14-952):** Use at a final assay concentration of 30nM. Prepare a 240nM stock in 1x reaction buffer and add 2.5µl of stock per assay point.
5. **ATM, active:** Dilute with 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol, 5mM DTT, 1mg/ml BSA. Use 0–50ng per assay point.
6. **Stop Solution:** 12.5mM HEPES pH8.0, 0.005% Brij-35, 0.5% Glycerol, 250mM EDTA.
7. **Detection Mix:** 50mM HEPES pH7.0, 150mM NaCl, 267mM KF, 0.1% sodium cholate, 0.01% Tween 20, 0.0125% sodium azide, anti-phospho-p53 (Ser15)-K (CisBio 61P08KAE) 0.42ng/well, and anti-GST-d2 (CisBio 61GSTDLA) 25ng/well.

Suggested Assay Procedure (384 well plate format):

The volumes detailed below are suitable for a 384-well plate (e.g. Corning Costar 3573) using a 20µL reaction volume (30µL stopped volume).

Assay Procedure

1. Add 10µl of 1 x reaction buffer per assay to wells.
2. Add 2.5µl of pre-diluted p53 (expressed in *E.coli*).
3. Add 2.5µl (0–50ng) ATM.
4. Add 5µl of ATP mixture to initiate the reaction.
5. Incubate for 30 minutes at room temperature.
6. Stop the reaction by adding 5µl of the 12.5mM HEPES pH8.0, 0.005% Brij-35, 0.5% Glycerol, 250mM EDTA.
7. Add 5µL Detection Mix
8. It is recommended that the plate is sealed to minimize reduction in reaction volume. It is recommended that the plate is read after an overnight incubation following the termination of the reaction and addition of the Detection Mix.
9. Measure HTRF ratio on an appropriate microplate reader according to the following parameters:

Excitation	330 - 380nm
Emission	665 - 667.5nm and 620 - 635nm
Counting Delay	50µsec
Counting window	(integration time) 400µsec

Refer to your instrument manufacturer for further guidance on measurement parameters recommended for HTRF.

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Calculation:

HTRF Ratio is calculated as follows:

$$HTRF\ Ratio = \left(\frac{Emission\ at\ 665nm}{Emission\ at\ 620nm} \right) \times 10000$$

Reviewed and approved by site quality representative.