

ABL2, Active

Recombinant protein expressed in Sf9 cells

Catalog # A04-11H

Lot # F712-2

Product Description

Recombinant human ABL2 (38-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is [NM_005158](#).

Gene Aliases

ARG; ABLL

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

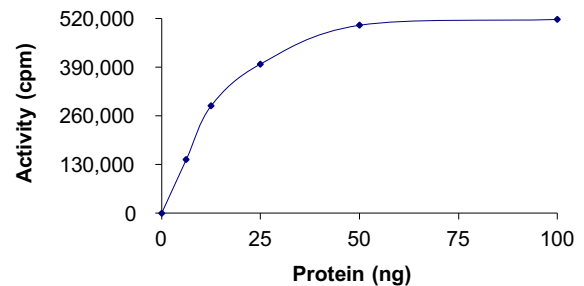
Scientific Background

ABL2 (or ARG) is a nonreceptor cytoplasmic tyrosine kinase which is closely related to but distinct from ABL1. The similarity of ABL1 and ABL2 includes the tyrosine kinase domains and extends amino-terminal to include the SH2 and SH3 domains. ABL2 is involved in translocation with the ETV6 gene in human leukemia and has an altered expression in several human carcinomas (1). Two isoforms of ABL2 with different N-termini (1A and 1B) have been identified. The C-terminal domain of ABL2 contains two F-actin-binding sequences that perform a number of actions related to cell morphology and motility by interacting with actin filaments (2).

References

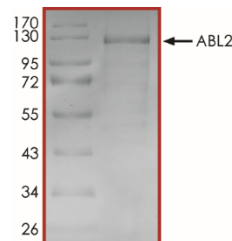
1. Barila, D. et al : An intramolecular SH3-domain interaction regulates c-Abl activity. Nature Genet. 18: 280-282, 1998.
2. Griesinger, F. et al: Identification of an ETV6-ABL2 fusion transcript in combination with an ETV6 point mutation in a T-cell acute lymphoblastic leukaemia cell line. Br J Haematol. 2002 Nov;119(2):454-8.

Specific Activity



The specific activity of ABL2 was determined to be **1050 nmol /min/mg** as per activity assay protocol.

Purity



The purity of ABL2 was determined to be **>70%** by densitometry, approx. MW **132kDa**.

ABL2, Active

Recombinant protein expressed in Sf9 cells

Catalog #	A04-11H
Specific Activity	1050 nmol/min/mg
Lot #	F712-2
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: A04-11H)

Active ABL2 (0.1µg/µl) diluted with Kinase Dilution Buffer VII (Catalog #: K27-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active ABL2 for optimal results).

Kinase Dilution Buffer VII (Catalog #: K27-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA and 5% glycerol solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: A02-58)

Abltide synthetic peptide substrate (EAIYAAPFAKKK) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active ABL2, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active ABL2 (Catalog #A04-11H)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #A02-58)
 - Component 3.** 5µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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