Recombinant Human c-Abl His-tag

Catalog Number: 11091-AL

DESCRIPTION

Source	*E. coli*-derived human c-Abl protein Gly227-Gln513, with an N-terminal Met and 6-His tag Accession # P00519.4
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	34 kDa

SPECIFICATIONS

SDS-PAGE	33-35 kDa, under reducing conditions
Activity	Measured by its ability to phosphorylate the Abl peptide substrate EAIYAAPFAKKK. The specific activity is >200 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Universal Kinase Activity Kit (Catalog # EA004)
- 10X Assay Buffer (supplied in kit): 250 mM HEPES, 1.5 M NaCl, 100 mM MgCl2, 100 mM CaCl2, pH 7.0
- Recombinant Human ABL-1 (rhABL-1) (Catalog # 11091-AL)
- Substrate: Abltide peptide (SignalChem, Catalog # A02-58), 1 mg/mL stock in 20 mM Tris, pH 7.5
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer by diluting 10X stocks 10 fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Universal Kinase Activity Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Prepare Reaction Mixture containing 0.4 mM ATP (supplied in kit) and 0.2 mg/mL Abltide peptide in 1X Assay Buffer.
5. Dilute rhABL-1 to 33.35 ng/µL in 1X Assay Buffer.
6. Dilute Coupling Phosphatase 4 (supplied in kit) to10 ng/µL in 1X Assay Buffer.
7. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
8. Load 15 µL of the 33.35 ng/µL rhABL-1 into empty wells of the same plate as the curve. Include a Control containing 15 µL of 1X Assay Buffer.
9. Add 10 µL of 10 ng/µL Coupling Phosphatase 4 to wells containing enzyme and Control, excluding the standard curve.
10. Add 25 µL of Reaction Mixture to the wells, excluding the standard curve.
11. Incubate sealed plate at room temperature for 10 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.
** The coupling rate is 0.475 under these conditions.

Final Assay Conditions

Per Reaction:

- rhABL-1: 0.5 µg
- Coupling Phosphatase 4: 0.1 µg
- ATP: 0.2 mM
- Abltide peptide: 5 µg

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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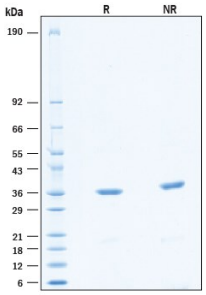
DATA

SDS-PAGE



Recombinant Human c-Abl His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human c-Abl His-tag Protein (Catalog # 11091-AL) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at ~33-35 kDa.

BACKGROUND

Tyrosine protein kinase ABL1 (Abelson murine leukemia viral oncogene homolog 1) is a ubiquitously expressed, magnesium-dependent, cytosolic member of the ABL subfamily of non-receptor protein tyrosine kinases (1). Human nonreceptor tyrosine kinases share a conserved domain structure that contributes to activity regulation and substrate specificity in many key processes linked to cell growth and survival (2-4). In addition, ABL1 contains domains unique to the ABL1 paralog that enable its function in DNA binding and DNA-damage response. ABL1 is composed of an N-terminal glycine myristoyl group that blocks the surface pocket of the kinase domain, a cap that stabilizes an inactive conformation, SH3 and SH2 domains that impose a locked inactive state, a protein kinase region, three nuclear localization signals, a DNA-binding region, and C-terminal nuclear export signal motifs, and actin F-binding domain (5-9). Activity is regulated through disruption of autoinhibitory interactions and several phosphorylation events that increase or decrease activity or promote function through stabilization (4). The SH2 domain contributes to both catalytic activity and target site specificity as has been shown with domain swapping (10). In chronic myelogenous, acute myeloid, and acute lymphoblastic leukemias, ABL1 is fused to BCR gene, resulting in deletion of the regulatory domains and production of a constitutively active tyrosine kinase (11). Several mutations in ABL1 lead to misregulation of activity in a variety of cancers (12). ABL1 kinase inhibition is used for therapeutic treatment in leukemic cancers and requires further development of drugs to address ABL1-mutation conferred resistance (12,13).

References:

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