



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

Product Information

Protein Kinase A from bovine heart

Catalog Number **P5511**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

EC 2.7.11.11 (Formerly 2.7.1.37)
Synonyms: PKA; cAMP-dependent protein kinase;
ATP:protein phosphotransferase (cAMP-dependent)

Product Description

Protein Kinase A (PKA) catalyzes the transfer of the terminal phosphate of ATP to threonine or serine residues in a variety of protein substrates. The enzyme is composed of two subunit types: a catalytic subunit and a regulatory subunit. In the absence of cAMP, the two subunits are bound to each other and no catalysis can take place. In the presence of cAMP, the regulatory subunit binds cAMP, thus releasing the catalytic subunit.¹

A reducing agent such as dithiothreitol is necessary to maintain reduced thiol groups and enzyme activity. The presence of a divalent cation is an absolute requirement for protein kinase A activity. Mg^{2+} at 10 mM yields best results; Co^{2+} can partially substitute for Mg^{2+} , but 10 mM Ca^{2+} inhibits protein kinase activity,² even in the presence of magnesium ions. The enzyme is inhibited by H-7, H-8, HA 1077, genestein, hypericin, staurosporine, and HA 100.³

The product is lyophilized from a solution containing: 5–10% potassium phosphate buffer, pH 7.5, 5–10% EDTA, and 80–90% protein (biuret assay).

Sigma quality control process improvement has developed a revised enzymatic activity assay procedure.

New Unit Definition: One unit will transfer 1.0 picomole of phosphate from $\gamma\text{-}^{32}\text{P}\text{-ATP}$ to hydrolyzed, partially dephosphorylated casein per minute at pH 6.5 at $30\text{ }^{\circ}\text{C}$ in the presence of cAMP.

Original Unit Definition was: One unit will transfer 1.0 picomole of phosphate from $\gamma\text{-}^{32}\text{P}\text{-ATP}$ to hydrolyzed, partially dephosphorylated casein per minute at pH 6.5 at $30\text{ }^{\circ}\text{C}$ in the presence of 0.006 mM cAMP.

400 new units are approximately equal to 1000 original units.

Specific activity: ~ 0.5 unit/ μg protein

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

It is recommended that a 1 mg/ml or greater stock solution be prepared in water or 0.5 mM citrate buffer, pH 6.5, and stored in aliquots at $-20\text{ deg. }^{\circ}\text{C}$.

Storage/Stability

Store the product at $-20\text{ }^{\circ}\text{C}$. The dry solid is shipped at ambient temperature with minimal loss in activity. When stored at $-20\text{ }^{\circ}\text{C}$ with desiccant, the protein will lose <10% activity per year.

References

1. Beavo, J.A. *et al.*, *Methods in Enzymology*, **38**, 299 (1974).
2. Shoji, S. *et al.*, *Biochemistry*, **22**, 3702-3709 (1983).
3. Handbook of Enzyme Inhibitors, 2nd ed., part A, Zollner, H., VCH (Weinheim, Germany: 1993), pp. 419-420.

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