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Product Information

Protein Kinase A Catalytic Subunit from bovine heart

Catalog Number **P2645**
Storage Temperature –20 °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] In the presence of cAMP, the catalytic subunit exists as a monomer of 40,862 Da (amino acid sequence),[2,3] but on SDS-PAGE the apparent molecular mass is 43,000 Da.

pI:[4] 7.01, 7.48, and 7.78 (three isozymes)

pH optimum:[1] 7.0–7.8

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,[3] even in the presence of magnesium ions.

The catalytic subunit product is prepared from the holoenzyme by the addition cAMP and then separation of the catalytic and regulatory subunits.

The product is lyophilized from a solution containing approximately: 80% sucrose, 19% potassium phosphate buffer, pH 6.7, 0.0625% 2-mercaptoethanol (2-ME), 0.002% EDTA, 0.016% dithiothreitol (DTT), and ≤1% protein. The lyophilized product may contain traces of DTT or 2-ME.

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 γ-^{32}P-ATP to hydrolyzed, partially dephosphorylated casein per minute at pH 6.5 at 30 °C.

Original Unit Definition was: One unit will transfer 1.0 picomole of phosphate from ATP to a synthetic substrate per minute at pH 7.4 at 30 °C.

400 new units are equal to ~1,000 original units.

Specific activity: 9–11 units/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
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 at 10 mM,[3] even in the presence of magnesium ions.

It is recommended to reconstitute the product in water containing 6 mg/ml dithiothreitol (DTT) at a concentration of 0.05 mg protein/ml and let stand at room temperature for 10 minutes prior to use. Do not reconstitute with water only or activity will be rapidly lost. This solution will remain active for 72 hours at 2–8 °C. Activity drops gradually, with most activity lost after a week.

Table 1.
Solution Activity Studies

Solvent	Protein Concentration	Temperature	Change after 8 days
water	50 µg/ml	–15 °C	40% loss when thawed
water	50 µg/ml	+ 4 °C	24% loss
water + DTT	50 µg/ml	–15 °C	98% loss when thawed
water + DTT	50 µg/ml	+ 4 °C	15% loss

If DTT interferes with a user's system, an aqueous solution of catalytic subunit without DTT may be used for one day. Alternatively, use 2-ME at ~0.04 mM in a protein solution of 0.05 mg/ml.

Snap-freezing aliquots without DTT, then lyophilizing the aliquots, will give better results than simply freezing solutions. Even under ideal conditions, over 50% activity is lost when the protein is frozen. Samples should be dried completely to retain any activity.

Storage/Stability
Store the product at –20 °C.

The dry solid is shipped at ambient temperature with minimal loss in activity. When stored at –20 °C with desiccant, the protein will lose <10% activity per year. However, the preparation is highly hygroscopic and moisture uptake will cause significant loss of activity. Samples stored at 37 °C for 7 days retained an average of 50% activity.

References

1. Beavo, J.A. et al., *Methods in Enzymology*, **38**, 299 (1974).
2. Taylor, S.S. *et al*., *Ann. Rev. Biochem.*, **59**, 971 (1990).
3. Shoji, S. et al., *Biochemistry*, **22**, 3702-3709 (1983).
4. Peters, K.A. *et al*., *Biochemistry*, **16**, 5691-5696 (1977) cited by Righetti in *J. Chrom.*, **220**, 115-194 (1981).

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