

Dephosphorylated α -Casein from Bovine Milk

Introduction

Dephosphorylated α -Casein from Bovine Milk is a lyophilized product prepared from Bovine milk α -casein through enzymatic dephosphorylation. α -Casein is one of the major caseins in bovine milk and naturally contains multiple phosphorylation sites (phosphoserine clusters). These phosphate groups play an important role in physiological processes such as the chelation of calcium ions by casein. Treatment with enzymes such as alkaline phosphatase gradually removes the phosphate groups from the protein. This product is a partially dephosphorylated form, typically showing two bands on Phosbind SDS-PAGE—dock dock one band corresponding to residual phosphorylated α -casein and the other to fully dephosphorylated α -casein.

The degree of dephosphorylation of this product is no less than 80%. It is recommended as a positive control for Phosbind SDS-PAGE, used in conjunction with the new phosphorylation detection tool Phosbind Acrylamide (Cat. No. F4002), to verify that the Phosbind SDS-PAGE detection system is functioning properly. The product is supplied as a lyophilized powder in sizes of 1 mg and 10 mg. Taking the 1 mg size as an example, using 5 μ g per well allows for approximately 200 experiments.

Protocol

1. Prepare working solution: Add an appropriate amount of water to a certain quantity of dephosphorylated α -casein to dissolve and prepare the working solution. For example, add 1 mL of water to 1 mg of product to prepare a 1 μ g/ μ L working solution.

***Note:** 1. Before using the product, centrifuge briefly to collect the lyophilized powder at the bottom of the tube, then proceed with subsequent operations. 2. Storage conditions: Lyophilized powder is recommended to be stored at -20°C , where it is stable for up to 1 year; working solution (after reconstitution) is recommended to be stored at -20°C , where it is stable for 1–3 months. 3. It is recommended to aliquot the reagent to avoid repeated freeze-thaw cycles.

2. Prepare separating gels: Prepare a standard 12% SDS-PAGE separating gel and a 12% Phosbind SDS-PAGE separating gel (containing 100 μ M Phosbind, with a Phosbind: Mn^{2+} molar ratio of 1:2).
3. Load samples: Add an appropriate amount of Dephosphorylated α -Casein from Bovine Milk (from step 1) to a single well as a positive control (e.g., 3 μ g for SDS-PAGE, 5 μ g for Phosbind SDS-PAGE). Add an appropriate amount of protein marker to an adjacent well. It is recommended to use a prestained protein marker compatible with Phosbind products, namely Prestained Protein Marker (Triple color, EDTA free, 10-250 kDa) (Cat. No. F4005).
4. Electrophoresis conditions: For a single gel, run at a constant current of 25 mA for 60 min; for two gels, run at

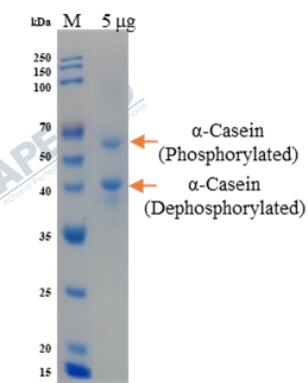
a constant current of 60 mA for 60 min. Electrophoresis parameters may be adjusted according to specific experimental results.

5. CBB staining: After electrophoresis, stain the gel with Coomassie Brilliant Blue (CBB) to visualize the results. A rapid protein staining solution (CBB staining method, Cat. No. B8226) is recommended.

6. Expected results (reference):

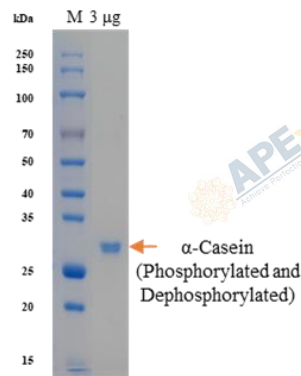
- Right panel (12% separating gel without Phosbind): A distinct band is observed between 25–35 kDa, containing both phosphorylated and dephosphorylated α -casein.
- Left panel (12% separating gel with Phosbind): Two distinct bands are observed between 40–75 kDa, with the upper band representing phosphorylated α -casein and the lower band representing dephosphorylated α -casein.

Phosbind SDS-PAGE



Gel: 12%
Phosbind: 100 μ M
Phosbind : Mn^{2+} = 1 : 2

SDS-PAGE



Gel: 12%

Note

1. Storage and shipping: Store at -20°C (stable for 1 year). Ship on dry ice.
2. This product is for scientific research use only.

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