CREATIVE BIOMART®
Assay Kit

Actin Binding Protein Assay Kit: human platelet actin

Product Information

Cat.No. Kit-0045

Product Overview

The Non-Muscle Actin Binding Protein Spin-Down Biochem Kit is an extremely quick and economical way to obtain an answer concerning binding affinity for monomer (G-) or polymer (F-) actin. If you are new to the field you may not know that actin requires ATP and a divalent cation for stability. Without this knowledge it is easy to obtain incorrect data, which can lead to inappropriate experimental interpretation. This kit is designed to guide you through the process of studying actin binding proteins. The Actin Binding Protein Spin-Down Biochem Kit provides G-or F-actin plus positive (-actinin) and negative (Bovine Serum Albumin, BSA) binding control proteins. Actin binding occurs when there is an affinity for any site of actin. F-actin binding can be measured by using a spin down assay where centrifugation is used to separate F-actin from G-actin by differential sedimentation. F-actin binding proteins will co-sediment with actin filaments and form a pellet at the bottom of the centrifugation tube. F-actin severing proteins, G-actin binding proteins or non-actin binding proteins will stay in the supernatant. Actin severing proteins will result in more G-actin remaining in the supernatant compared to the negative control sample. This activity should be further tested by measuring F-actin length distributions before and after adding the test protein. G-actin binding proteins can be measured by adding the test protein to G-actin and inducing polymerization. If the test protein sequesters G-actin, more actin will remain in the supernatant compared with the control. Actin can exist in two forms: Globular subunit (G-actin) and Filamentous polymer (F-actin) (See Appendix 1 for more information about actin properties). Both forms of actin interact with a plethora of proteins in the cell. To date there are over 50 distinct classes of Actin-Binding Proteins (ABPs) and the inventory is still far from complete. Actin Binding Proteins allow the actin cytoskeleton to respond rapidly to cellular and extracellular signals and are integral to cytoskeletal involvement in many cellular processes. These include cell shape and motility, muscle contraction, intracellular trafficking, cell pathogenesis and signal transduction. A comprehensive review of the huge body of literature concerning the structure and functions of ABPs is beyond the scope of this general introduction and we direct the reader to several excellent review articles and references therein. In this introduction to ABPs we will briefly outline the major recognized classes of ABPs and the

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experimental procedures that are currently used to study ABP activity.

Size

30-100 assays

Applications

1. To determine whether a protein binds to filaments or monomers of actin.2. To determine whether a protein bundles F-actin.3. To test various conditions (e.g. pH optima) or requirements (e.g. divalent cation requirement) for binding to actin.

Storage

This kit should be stored at 4°C.

Kit Components

Reagent-Quantity-Description 1. Non-Muscle Actin: Lyophilized. >99% pure actin (43 kDa), 1 mg per tube. 2. actinin: Lyophilized. Positive control protein (116 kDa), 50 μg per tube. 3. BSA: Lyophilized. 3.4 mg/ml negative control protein (68 kDa). 3.4 mg per tube. 4. General Actin Buffer: Lyophilized. 5 mM Tris-HCl pH 8.0 and 0.2 mM CaCl2. 5. Actin Polymerization Buffer: Lyophilized. 500 mM KCl, 20 mM MgCl2, and 10 mM ATP. 10X strength. 6. F-actin Cushion Buffer: Liquid. 5 mM Tris-HCl pH 8.0, 50 mM KCl, 2 mM MgCl2, and 10% glycerol 7. ATP stock: Lyophilized. 100 mM ATP when resuspended EGTA solution BSEG-01 1 Liquid. 0.5 M EGTA MgCl2 solution BSMG-01 1 Liquid. 100 mM MgCl2.6H2O 8. Tris-HCl pH 7.5: Lyophilized. 100 mM Tris-HCl pH 7.5 when resuspended.9. Tris-HCl pH 6.5: Liquid. 1.0 M Tris-HCl pH 6.5.