コスモ・バイオ株式会社

Actin polymerization Biochem Kit™

Cat. # BK003

Product Uses Include

- To show quantitative / qualitative effects on actin polymerization by the addition of a tissue extract, an actin binding protein or compound.
- To show quantitative / qualitative effects on actin polymerization by addition of an F-actin nucleating protein, compound or extract.
- To show quantitative / qualitative effects on steady-state F-actin levels by addition of an F-actin severing protein, compound or tissue extract.
- To show quantitative / qualitative effects on actin depolymerization by addition of an actin binding protein, compound or tissue extract.

Introduction

The Actin Polymerization Biochem Kit™ is based on the enhanced fluorescence of pyrene conjugated actin that occurs during polymerization. The enhanced fluorescence that occurs when pyrene G-actin (monomer) forms pyrene F-actin can be measured in a fluorimeter to follow polymerization over time. Also, by using preformed pyrene F-actin, it is possible to follow depolymerization. Both cell/tissue extracts and purified proteins can be added to the reaction mixture to identify their effect on actin polymerization. The components of the kit can also be used separately for other actin based assays such as a spin-down assays to detect F-actin binding proteins (see also BK001) or size exclusion chromatography to identify G-actin binding proteins. See the About Actin page for more information on assays testing actin binding proteins.

While this kit comes with pyrene labeled skeletal muscle actin, it can also be used to study polymerization of other types of actin such as non-muscle actin (Cat. # APHL99) or cardiac actin (Cat. # AD99). Polymerization assays with these actins can be performed using a 10:1 ratio between the actin you want to study and the included pyrene actin

Kit contents

The kit contains enough materials for 30-100 assays depending on assay volume. The following reagents are included:

1. 5 x 1 mg Pyrene labeled actin (Cat. # AP05).
2. General Actin Buffer (Cat. # BSA01).
3. Actin Polymerization Buffer (Cat. # BSA02).
4. ATP 100mM (Cat. # BSA04).
5. Tris-HCl pH 7.5, 100 mM
6. Manual with detailed protocols and extensive troubleshooting guide.

Equipment needed

1. Fluorescence spectrophotometer (cuvette or 96-well plate) with 4-10 nm bandwidth at 365 nm excitation wavelength, and 4-10 nm bandwidth at 407 nm emission wavelength.
2. Small capacity (100-1000 µl) fluorescence spectrophotometer cuvette or 96-well plate.

Example results

The Actin Polymerization Biochem Kit™ was used to study the effects of Arp2/3 (Cat. # RP01) and the VCA domain of WASP (Cat. # VCG03) on actin polymerization rates. The Arp2/3 complex is an actin filament nucleator but has low nucleating/polymerizing activity on its own. The VCA domain of WASP is an activator of the Arp2/3 complex. Hence, when the Arp2/3 complex is mixed with the WASP VCA domain, these two exert a potent actin polymerizing activity (Fig. 1).

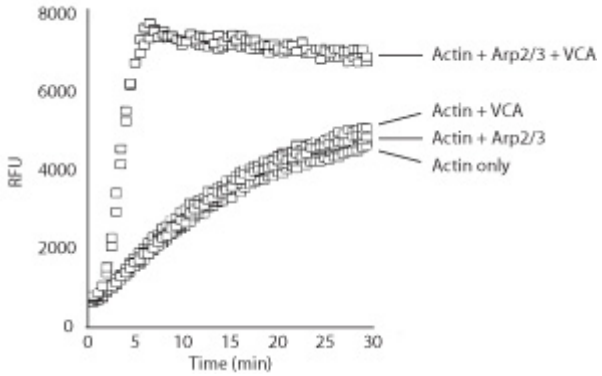

Figure 1. Actin polymerization stimulated by Arp2/3 complex and the VCA domain of WASP. Actin polymerization was measured using kit BK003. The addition of Arp2/3 complex or the VCA domain alone to actin has minimal effects on actin polymerization, while the combination of Arp2/3 and the VCA domain strongly stimulates the rate of actin polymerization.

Examples of publications where this product was used

Blader, I. J., Cope, M. J., Jackson, T. R., Profit, A. A., Greenwood, A. F., Drubin, D. G., Prestwich, G. D. and Theibert, A. B. (1999). GCS1, an Arf guanosine triphosphatase-activating protein in Saccharomyces cerevisiae, is required for normal actin cytoskeletal organization in vivo and stimulates actin polymerization in vitro. Mol. Biol. Cell 10, 581-596.

Fontao, L., Geerts, D., Kuikman, I., Koster, J., Kramer, D. and Sonnenberg, A. (2001). The interaction of plectin with actin: evidence for cross-linking of actin filaments by dimerization of the actin-binding domain of plectin. J. Cell Sci. 114, 2065-2076.

Kumar, N., Tomar, A., Parrill, A. L. and Khurana, S. (2004). Functional dissection and molecular characterization of calcium-sensitive actin-capping and actin-depolymerizing sites in villin. J. Biol. Chem. 279, 45036-45046.

Takamiya, R., Takahashi, M., Park, Y. S., Tawara, Y., Fujiwara, N., Miyamoto, Y., Gu, J., Suzuki, K. and Taniguchi, N. (2005). Overexpression of mutated Cu,Zn-SOD in neuroblastoma cells results in cytoskeletal change. Am. J. Physiol. 288, C253-259.

Zhai, L., Zhao, P., Panebra, A., Guerrerio, A. L. and Khurana, S. (2001). Tyrosine phosphorylation of villin regulates the organization of the actin cytoskeleton. J. Biol. Chem. 276, 36163-36167.