Actin Cytoskeleton and Focal Adhesion Staining Kit

For 100 Tests

Catalog No. FAK100

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

USA & Canada Phone: +1(800) 437-7500 Fax: +1-800-437-7502
www.millipore.com

Introduction

The actin cytoskeleton is a highly dynamic network composed of actin polymers and a large variety of associated proteins. The functions of the actin cytoskeleton is to mediate a variety of essential biological functions in all eukaryotic cells, including intra- and extra-cellular movement and structural support. To perform these functions, the organization of the actin cytoskeleton must be tightly regulated both temporally and spatially. Many proteins associated with the actin cytoskeleton are thus likely targets of signaling pathways controlling actin assembly. Actin cytoskeleton assembly is regulated at multiple levels, including the organization of actin monomers (G-actin) into actin polymers and the superorganization of actin polymers into a filamentous network (F-actin – the major constituent of microfilaments) [1]. This superorganization of actin polymers into a filamentous network is mediated by actin side-binding or cross-linking proteins [2, 3, 4]. The actin cytoskeleton is a dynamic structure that rapidly changes shape and organization in response to stimuli and cell cycle progression. Therefore, a disruption of its normal regulation may lead to cell transformations, hence cancer. Transformed cells have been shown to contain less F-actin than untransformed cells and exhibit atypical coordination of F-actin levels throughout the cell cycle [5]. Orientational distribution of actin filaments within a cell is, therefore, an important determinant of cellular shape and motility.

Focal adhesion and adherens junctions are membrane-associated complexes that serve as nucleation sites for actin filaments and as cross-linkers between the cell exterior, plasma membrane and actin cytoskeleton [6]. The function of focal adhesions is structural, linking the ECM on the outside to the actin cytoskeleton on the inside. They are also sites of signal transduction, initiating signaling pathways in response to adhesion. Focal adhesions consist of integrin-type receptors that are attached to the extracellular matrix and are intracellularly associated with protein complexes containing vinculin (universal focal adhesion marker), talin, α-actinin, paxillin, tensin, zyxin and focal adhesion kinase (FAK) [7,8].

Millipore's Actin Cytoskeleton and Focal Adhesion Staining Kit (Catalog Number FAK100) is a very sensitive immunocytochemical tool that contains fluorescent-labeled Phalloidin (TRITC-conjugated Phalloidin) to map the local orientation of actin filaments within cell and a monoclonal antibody to Vinculin that is very specific for the staining of focal contacts in cells. The kit also contains DAPI for the fluorescent labeling of nuclei.

Product Description and Storage

The Actin Cytoskeleton and Focal Adhesion Staining Kit consists of three components (TRITC-conjugated Phalloidin, anti-Vinculin and DAPI) for the immunofluorescent staining of actin filaments in the cytoskeleton, focal contacts as well as the nucleus of the cells.

Reagents and materials supplied in the Actin Cytoskeleton and Focal Adhesion Staining Kit are sufficient for 100 tests (including controls).

When stored at 2-8ºC, the kit components are stable up to the expiration date. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the expiration date.

Kit Components

1. Vinculin Monoclonal Antibody, purified clone 7F9 (Part No. 90227). One vial containing 100 µL at 1 mg/mL.
2. TRITC-conjugated Phalloidin (Part No. 90228). One vial containing 15 µg lyophilized TRITC-conjugated Phalloidin.
3. DAPI (Part No. 90229). One vial containing 100 µL at 0.1mg/mL.

Materials Required But Not Supplied

1. Fixative, e.g. 4% paraformaldehyde
2. Permeabilizing reagent, e.g. 0.1% Triton X-100
3. Blocking solution (1% BSA in 1x PBS)
4. Fluorescent-labeled anti-mouse secondary antibody (For example Millipore Cat. No. AP124F)
5. 1x wash buffer (e.g. 1x PBS containing 0.05% Tween-20)
6. Antifade mounting solution (For example: Millipore Cat. No. 5013)
7. Glass slides and coverslips

Preparation of Reagents

TRITC-conjugated Phalloidin. To prepare a stock solution: Resuspend one vial of TRITC-conjugated Phalloidin (15 µg) into 250 µL of methanol. Store at -20°C upon reconstitution.

Assay Protocol

1. Passage cells and culture in suitable media until approximately 50-60% confluent.
2. Fix cultured cells with 4% paraformaldehyde in 1x PBS for 15-20 minutes at room temperature.
3. Wash twice with 1x wash buffer.
4. Permeabilize cells with 0.1% Triton X-100 in 1x PBS for 1-5 minutes at room temperature.
5. Wash twice with 1x wash buffer.
6. Apply blocking solution for 30 minutes at room temperature.
7. Dilute primary antibody (Anti-Vinculin) to a working concentration in blocking solution, and incubate for 1 hour at room temperature.

   Note: *Optimal primary antibody titer must be determined prior to performing immunocytochemistry. Recommended dilution for anti-Vinculin is 1:100-1:500.*
8. Wash three times (5-10 minutes each) with 1x wash buffer.
9. Dilute secondary antibody (Gt x Ms, FITC-conjugated, for example Millipore Cat. No. AP124F) in 1x PBS just before use and incubate for 30-60 minutes at room temperature. For double labeling TRITC-conjugated Phalloidin can be incubated simultaneously with the secondary antibody for 30-60 minutes at room temperature.

   Note: *Optimal secondary antibody titer must be determined prior to performing immunocytochemistry. Optimal dilution for TRITC-conjugated Phalloidin is 1:100 -1:1,000.*
10. Wash three times (5-10 minutes each) with 1x wash buffer.
11. OPTIONAL: Following this washing step, nuclei counterstaining can be performed by incubating cells with DAPI for 1-5 minutes at room temperature, followed by washing cells three times (5-10 minutes each) with 1x wash buffer.

    Note: *Optimal DAPI titer must be determined prior to performing counterstaining. Recommended starting dilution is 1:1,000.*
12. If immunocytochemistry was performed on a 24-well plate, cells should be covered with 1x PBS prior to visualization to prevent cells from drying out. However, if cells are stained on a coverslip it can be mounted on a slide by using antifade mounting solution.
13. Fluorescence images can be visualized with a fluorescence microscope. *NOTE: Be sure to use the correct filter for visualizing fluorescent-labeled cells.*

Example Results

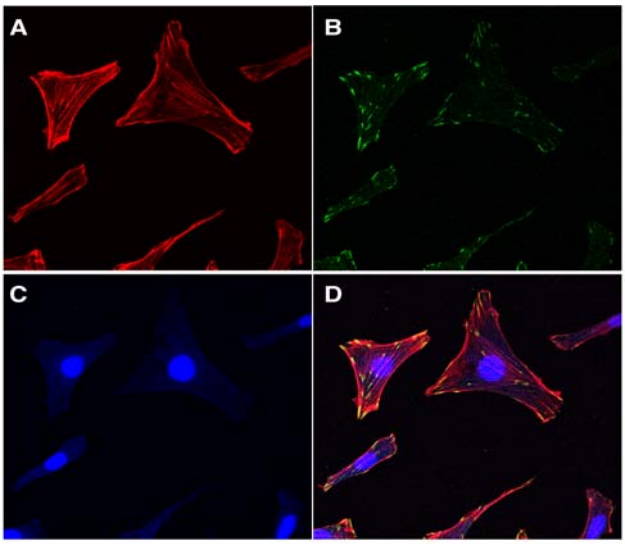

Figure 1. Confocal fluorescence microscopy of focal adhesion and actin cytoskeleton in NIH-3T3 cells. **(A)** F-actin was detected using TRITC-conjugated Phalloidin, **(B)** focal contacts were revealed using anti-Vinculin monoclonal antibody and a FITC-conjugated secondary antibody, **(C)** nuclear counterstaining revealed with DAPI, **(D)** monochrome images of TRITC-conjugated Phalloidin (A), anti-Vinculin (B) and DAPI (C) were overlaid and displayed in pseudocolor.

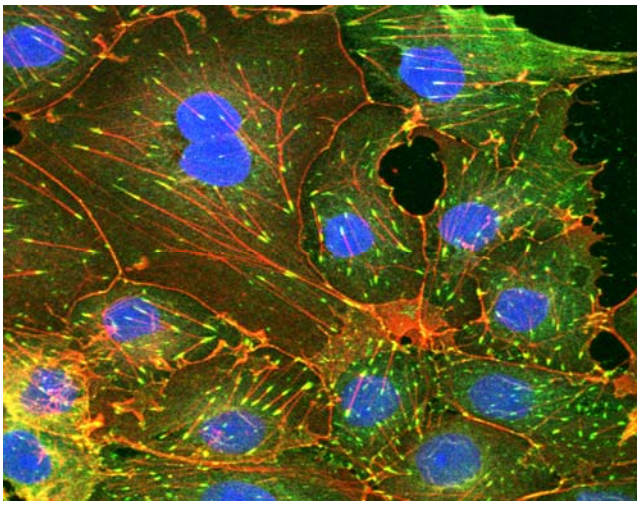

Figure 2. Confocal fluorescence microscopy of focal adhesion and actin cytoskeleton in COS-7 cells revealed with triple labeling using TRITC-conjugated Phalloidin (staining F-actin), anti-Vinculin (focal contacts) and DAPI (nuclei).

References

1. Bretscher A et al. (1994). *J Cell Biol* **126**:821-825.
2. Dubreuil RR. (1991). *BioEssays* **13**:219-226.
3. Matsudaira P. (1991). *Trends Biochem Sci* **16**:87-92.
4. Matsudaira P. (1994). *Semin Cell Biol* **5**:165-174.
5. Rao JY et al. (1990). *Cancer Res* **50**:2215-2220.
6. Yamada KM, Geiger B. (1997). *Curr Opin Cell Biol* **9**:76-85.
7. Burridge K et al. (1990). *Cell Diff Dev* **32**:337-342.
8. Turner CE, Burridge K. (1991). *Curr Opin Cell Biol* **3**:849-853.

Warranty

Millipore Corporation ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the Company all monies paid for such applicable Product. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

Cat No. FAK100

May 2009
Revision C: 41341