BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Alexa Fluor® 488 Mouse anti-Actin

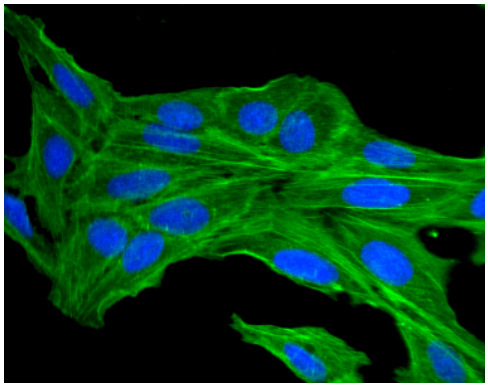
Product Information

Material Number:	**558623**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	C4/actin
Immunogen:	Chicken gizzard muscle Actin
Isotype:	Mouse IgG1, κ
Reactivity:	Tested: Human Reported: Dictyostelium discoideum, Physarum polycephalum Confirmed by western blot using purified antibody (Cat. No. 612656 or 612657): Chicken, Dog, Mouse, Rat, Green Algae
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Changes in cellular morphology, adhesion, and motility occur through the reorganization of the actin cytoskeleton. This reorganization of actin filaments results from interactions between actin and actin-binding proteins. Actin is a 42-kDa protein that is known as G-actin in its monomeric form. Polymerization of G-actin monomers leads to the generation of flexible filaments, 5-9 nm in diameter, called F-actin. F-actin may be organized in linear bundles called stress fibers or in two-dimensional networks. The latter are highly concentrated beneath the plasma membrane and form the actin cortex. Regulation of actin cytoskeletal dynamics occurs through actin-binding proteins. These proteins bind to G- and/or F-actin and regulate various aspects of actin cytoskeletal dynamics, such as polymerization and depolymerization of actin, cross-linking of actin filaments into bundles, interaction of actin-based structures with membranes and other cytoskeletal elements, and locomotion of actin-based structures. Thus, the actin cytoskeleton is a complex matrix consisting of G- and F-actin along with the multitude of interactions between these actin forms and a variety of different types of actin-binding proteins.

The C4 monoclonal antibody reacts with all known isoforms of actin in vertebrate muscle and non-muscle cells.



Immunofluorescent staining of human cell lines. *U-2 OS cells (ATCC HTB-96) were cultured, fixed, permeabilized with cold methanol, stained with Alexa Fluor® 488 Mouse anti-Actin (pseudo-colored green), and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 855 Bioimager System with a 20x objective and merged using BD Attovision™ software. This antibody also stains A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells. Triton X-100 is not recommended as a permeabilization agent with this antibody.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

Recommended Assay Procedure

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells by adding 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm

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Buffer III (Cat. No. 558050) to each well and incubating for 5 minutes at RT.
4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (3% FBS in 1× PBS) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well and incubating for 30 minutes at RT.
6. Remove the blocking buffer, dilute the antibody conjugate 1:10 in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody conjugate, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
9. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Hanstein C, Lange U, Schneider-Poetsch HA, Grolig F, Wagner G. Detection of actin and localization of phytochrome in the green alga Mougeotia by monocloanl antibodies. *Acta Histochem Suppl.* 1991; 41:223-230. (Clone-specific)
Lesssard JL. Two monoclonal antibodies to actin: one muscle selective and one generally reactive. *Cell Motil Cytoskeleton.* 1988; 10(3):349-362. (Immunogen)
Pantaloni D, Le Clainche C, Cartier M-F. Mechanism of Actin-Based Motility. *Science.* 2001; 292:1502-1506. (Biology)