Alexa Fluor® 647 Mouse Anti-Fibronectin

Product Information

Material Number: 563098
Alternate Name: FN; LETS
Size: 50 tests
Vol. per Test: 5 µl
Clone: 10/Fibronectin
Immunogen: Human Fibronectin
Isotype: Mouse IgG1, κ
Reactivity: QC Testing: Human
Confirmed in Development: Mouse, Rat, Dog, Chicken, Bovine

Storage Buffer: Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The 240-kDa dimeric fibronectin protein exists in two forms: a soluble protomer in body fluids and an insoluble multimer in the extracellular matrix. The latter is the primary functional form and creates a substrate for cell migration, a role which makes fibronectin vital to embryogenesis and wound response. Fibronectin mediates cytoskeletal organization, cell attachment, and cellular signaling through interactions with cellular receptors. Although various isoforms of fibronectin are derived by alternative splicing, they share a common N-terminus which is a critical region for cell surface binding in an initial step of multimer assembly. Further polymerization steps are regulated by fibronectin/integrin interactions and result in generation of the complex fibrils that constitute the fibronectin matrix.

Preparation and Storage

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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Flow cytometric analysis of fibronectin in human mesenchymal stem cells (MSC). MSC (Lonza), passage 6, were dissociated and fixed in BD Cytofix™ Fixation Buffer (Cat. No. 554655), and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were stained with either Alexa Fluor® 647 Mouse IgG1, κ isotype control (dashed line, Cat. No. 557714) or Alexa Fluor® 647 Anti-Fibronectin monoclonal antibody (solid line) at matched concentrations. Histograms were derived from gated events based on light scattering characteristics of MSC. Flow cytometry was performed on a BD LSRFortessa™ II flow cytometry system. BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885) is also suitable for permeabilization.

Immunofluorescent analysis of fibronectin in human mesenchymal stem cells (MSC). MSC (Lonza), passage 6, were fixed in BD Cytofix™ Fixation Buffer (Cat. No. 554655), permeabilized with 0.1% Triton™ X-100, and stained with Alexa Fluor® 647 Mouse Anti-Fibronectin monoclonal antibody (pseudo-colored red) at 1.2 µg/ml. Cell nuclei were stained with DAPI (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software. BD Phosflow™ Perm Buffer III (Cat. No. 558050) is also suitable for permeabilization.

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Application Notes

Application

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<thead>
<tr>
<th>Intracellular staining (flow cytometry)</th>
<th>Routinely Tested</th>
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<tbody>
<tr>
<td>Bioimaging</td>
<td>Tested During Development</td>
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<tr>
<td>Immunofluorescence</td>
<td>Tested During Development</td>
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Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
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<td>554655</td>
<td>Fixation Buffer</td>
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<tr>
<td>558050</td>
<td>Perm Buffer III</td>
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<tr>
<td>557885</td>
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<td>557714</td>
<td>Alexa Fluor® 647 Mouse IgG1 κ Isotype Control</td>
<td>100 tests</td>
<td>MOPC-21</td>
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</table>

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use $1 \times 10^6$ cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. 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.
7. Triton is a trademark of the Dow Chemical Company.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References