

## Reagents Provided

**Alexa Fluor® 488-conjugated mouse monoclonal anti-human/mouse Myogenin:** Supplied as 25 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 671038

**Isotype:** mouse IgG<sub>1</sub>

## Reagents Not Provided

**Flow Cytometry Fixation Buffer** (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

**Flow Cytometry Permeabilization/Wash Buffer I (1X)** (Catalog # FC005) or other saponin-containing saline buffer.

## Storage

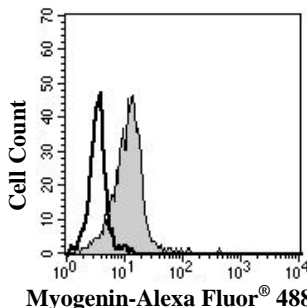
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing Myogenin within a population and qualitatively determine the density of intracellular Myogenin by flow cytometry.

## Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with *E. coli* derived recombinant human Myogenin (Ser124 - Asn224; Accession # P15173). The IgG fraction of the tissue culture supernatant was purified by Protein A/G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Intracellular expression of Myogenin is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.



C2C12 mouse cells were stained with Alexa Fluor®488-conjugated anti-human/mouse Myogenin (Catalog # IC6686G, filled histogram) or Alexa Fluor®488-conjugated isotype control (Catalog # IC002G, open histogram).

## Legal

This product is provided under an agreement between Life Technologies Corporation and R&D Systems, Inc. and the manufacture, use, sale or import of this product is subject to one or more US patents and corresponding non-US equivalents, owned by Life Technologies Corporation and its affiliates. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) to resell, sell, or otherwise transfer this product or its components to any third party, or for any other commercial purpose. Life Technologies Corporation will not assert a claim against the buyer of the infringement of the above patents based on the manufacture, use or sale of a commercial product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, Cell Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

## Background Information

Myogenin, also called MYF4, is a 34 kDa, 224 amino acid (aa) basic HLH transcription factor belonging to the myogenic regulatory factor (MRF) family. It induces fibroblastic differentiation to myoblasts and is most highly expressed early during skeletal muscle differentiation. Within the region used as an immunogen, human Myogenin shares 93% and 91% aa identity with mouse and rat Myogenin, respectively. Isoforms may exist that diverge at aa 155 (246 aa form) or that are truncated at aa 215.

## Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see [Reagents Not Provided](#)).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Up to  $1 \times 10^6$  cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 5 µL of conjugated antibody was added and cells were incubated for 30 minutes at room temperature **in the dark**.
6. Cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled mouse IgG<sub>1</sub> antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

**Warning:** Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.