

## Product Information

### Anti-Flotillin 1–Cy3™

produced in rabbit, affinity isolated antibody

Catalog Number **F8931**

#### Product Description

Anti-Flotillin 1–Cy3 is a Cy3 conjugate of Anti-Flotillin 1 antibody produced in rabbit using as immunogen a synthetic peptide corresponding to amino acid residues 413–428 of mouse flotillin 1 (GeneID 14251) conjugated to KLH. The corresponding sequence is identical in rat and differs by one amino acid in human. Anti-Flotillin 1. The Cy3 conjugate is prepared by conjugation of the affinity purified antibody to Cy3. The conjugate is purified by gel filtration to remove unbound Cy3 fluorophore.

Anti-Flotillin 1–Cy3 recognizes mouse, rat, and human flotillin 1. The product may be used for the detection and localization of flotillin 1 by direct immunofluorescence staining.

Flotillins are lipid raft-associated proteins involved in various cellular processes such as endocytosis, phagocytosis, neuronal regeneration and insulin signaling.<sup>1,2</sup> Lipid raft microdomains are glycosphingolipid- and cholesterol-rich membrane organized, dynamic structures connected to the cytoskeleton. Lipid raft domains are insoluble in non-ionic detergents, have a low buoyant density and contain proteins such as Src family kinases, glycosylphosphatidylinositol (GPI)-linked proteins, caveolins, sphingolipids and cholesterol. Lipid rafts have a central role in cellular organization, membrane trafficking and signaling events.<sup>3,4</sup> The flotillin protein family consists of two proteins, flotillin 1 and 2, also named reggie-2 and -1, respectively. Flotillins belong to a larger family of integral membrane proteins that contain an evolutionarily conserved domain called the prohibitin homology (PHB) domain or SPFH domain (stomatin/prohibitin/flotillin/HflK/C domain). It has been suggested that the PHB domain constitutes a lipid recognition motif which can target proteins to the plasma membrane.<sup>2,4</sup> The association of flotillin 1 to

the plasma membrane depends on palmitoylation in a conserved Cys<sup>34</sup> residue, whereas the association of flotillin 2 depends on both myristolation and palmitoylation.<sup>2,5</sup>

The flotillin proteins are conserved and ubiquitously expressed. Flotillin 1 and 2 show a complementary tissue distribution. While flotillin 1 is mainly expressed in striated muscle tissues, adipose tissue and lung, flotillin 2 is much widely distributed, but is absent in skeletal muscle.<sup>6</sup> Flotillin 1 is mostly localized to the plasma membrane although it was reported to reside also in a specific population of endocytic intermediates. Flotillin 1 does not colocalize with endocytosed transferrin and caveolin-1, indicating that it could be associated with endocytosis via a pathway that is different from clathrin-coated pits and caveolin-1-positive caveolae.<sup>7</sup>

Flotillin 1 was found to specifically interact with the multivalent adaptor protein CAP, the Src family kinase Fyn and F-actin in lipid raft microdomains in adipocytes. Therefore, it was suggested that flotillin 1 may function as a molecular link between lipid rafts of the plasma membrane and a multimeric signaling complex at the actin cytoskeleton.<sup>4</sup> Flotillin 1 may be used as a marker protein for lipid raft microdomains.

#### Reagent

Supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: 1.0 - 3.0 mg/mL

Molar Ratio (F/P): 3-9

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage/Stability**

Store at –20 °C. For continuous use, the product may be stored at 2–8 °C for up to one month. For extended storage, freeze at –20 °C in working aliquots. Protect from prolonged exposure to light. Repeated freezing and thawing, or storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

**Product Profile**

Direct immunofluorescence: a working antibody concentration of 2–5 µg/mL is recommended using human HEK-293T or HeLa cells.

**Note:** In order to obtain the best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

**References**

1. Bickel, P.E., et al., *J. Biol. Chem.*, **272**, 13793–13802 (1997).
2. Neumann-Giesen, C., et al., *Biochem. J.*, **378**, 509–518 (2004).
3. Salzer, U., and Prohaska, R., *Blood*, **97**, 1141–1143 (2001).
4. Liu, J., et al., *J. Biol. Chem.*, **280**, 16125–16134 (2005).
5. Morrow, I.C., et al., *J. Biol. Chem.*, **277**, 48834–48841 (2002).
6. Volonte, D., et al., *J. Biol. Chem.*, **274**, 12702–12709 (1999).
7. Glebov, O.O., et al., *Nature Cell Biol.*, **8**, 46–54 (2006).

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