Human M-Cadherin/Cadherin-15 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF4096

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human M-Cadherin/Cadherin-15 in direct ELISAs and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human M-Cadherin/Cadherin-15 Val22-Ala606 Accession # P55291
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

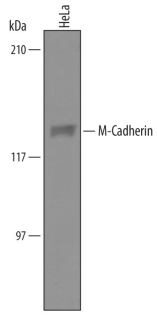
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

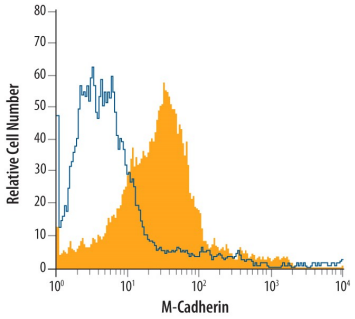
DATA

Western Blot



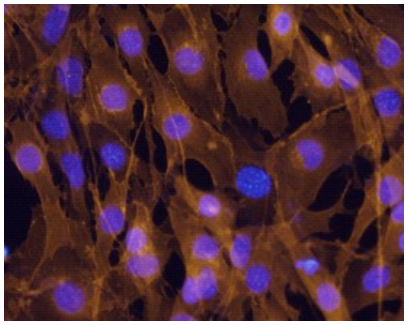
Detection of Human M-Cadherin/Cadherin-15 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human M-Cadherin/Cadherin-15 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4096) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for M-Cadherin/Cadherin-15 at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Flow Cytometry



Detection of M-Cadherin/Cadherin-15 in C2C12 Mouse Cell Line by Flow Cytometry. C2C12 mouse myoblast cell line was stained with Sheep Anti-Human M-Cadherin/Cadherin-15 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4096, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL010).

Immunocytochemistry



M-Cadherin/Cadherin-15 in C2C12 Mouse Cell Line. M-Cadherin/Cadherin-15 was detected in immersion fixed C2C12 mouse myoblast cell line using Sheep Anti-Human M-Cadherin/Cadherin-15 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4096) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the Northern-Lights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

M-Cadherin (M-CAD or Cadherin-15) is a 124 kDa type I transmembrane glycoprotein of the Cadherin superfamily of calcium-dependent homotypic adhesion molecules (1-4). Like other classical Cadherins, the 814 amino acid (aa) human M-CAD contains a signal sequence (21 aa), a propeptide (29 aa), an extracellular domain with five Cadherin domain repeats (ECD, 556 aa), a transmembrane segment (20 aa) and a cytoplasmic domain (188 aa) (1). A splice variant that diverges within the third Cadherin repeat has been sequenced. The Cadherin repeats are responsible for cell-cell adhesion by homophilic binding on opposing cells (1-4). Intracellularly, M-CAD binds β-catenin or plakoglobin (γ-catenin), which in turn bind α-catenin (4). M-CAD also binds p120 catenin (5). Connection of Cadherin/catenin complexes to the actin cytoskeleton is in question, but is possible through a linker (6). Connection to microtubules has been shown (7). M-CAD is present during early stages of skeletal muscle development and is thought to align myoblasts for fusion (8). It is also present in muscle satellite cells and participates in muscle regeneration (9). M-CAD is also expressed in the granule cell layer of the cerebellar glomerulus (10). Deletion of mouse M-CAD has little effect *in vivo*, most likely due to compensation by N-Cadherin (11). However, M-CAD upregulation and adhesion between myoblasts during induction of differentiation in vitro is required for their fusion (8, 12, 13). M-CAD activity is later downregulated by sequestering to caveoli, p120 catenin/RhoA-induced ubiquitination and/or cleavage by calpain-3. This terminates fusion and allows sarcomere formation (5, 12, 13). Human M-CAD ECD shows 88% aa identity with mouse, rat, or bovine and 85% aa identity with canine M-CAD ECD. M-CAD is an outlier among classical Cadherins, with 40% aa identity or less in the ECD (4).

References:

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