MONOCLONAL ANTIBODY REAGENTS DIAGNOSTIC FOR CDHR3, THE RHINOVIRUS-C CELLULAR RECEPTOR PROTEIN, DOMAINS EC1, EC2, EC5 AND EC6

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The Invention

UW-Madison researchers have developed and characterized nine murine monoclonal antibody (mAb) reagents that react uniquely with the EC1 (2x), EC2 (2x), EC5 (3x), and EC6 (2x) extracellular cadherin repeat domains of CDHR3.

Each mAb detects a unique protein sequence in its directed domain with high specificity and high affinity, making these reagents diagnostic and potent for CDHR3 detection in vitro or in vivo.

The inventors cloned, synthesized, refolded (conformationally) and purified bacterially produced EC1-2 and EC-5-6 proteins with protocols similar to those used in their previous studies. The inventors contracted with GenScript Biotech Corporation (Stock Code: 1548.HK) to develop panel of mAb materials reactive to their proteins. As they became available, GenScript sent preliminary mouse bleeds, and intermediate hybridoma supernatants for their screening (western, IP, IF, TEM labeling; vs various native and recombinant proteins) before they selected the 9 preferred reagents that were then finalized as viable hybridoma cell lines, and purified IgG materials, derived therefrom. Using the lab's specific recombinant derivatives of CDHR3 unique to each EC domain the inventors could readily identify each mAb's relative reactivity and EC specificity. Similarly, extensive IP, IF and Western assays with native (cultured cells, transformed cells, transduced cells, air-liquid-interface (ALI) airway epithelial cells, human biopsy fixed cells) and recombinant-isolated proteins, characterized each mAb's activities and usefulness in typical experimental protocols. The glycosylation status of EC domains does not affect mAb reactivity for any of the tested reagents, and none of them, even for EC1, interfere with RV-C15 binding to susceptible cultured cells or ALI.

The inventors are in possession of viable hybridoma (frozen aliquots) cells and purified mAbs. The inventors can direct GenScript to send additional samples of the hybridomas wherever we request. Although the inventors characterized 9 independent mAbs for their panel, their ongoing research has settled on 4 preferred iterations, one for each of the targeted domains; these (with asterisks) seem to have slightly higher target affinities, require lower concentrations of mAb for signal detection (across all platforms) and tend to have marginally "cleaner" background detection of superfluous protein. The inventors suggest the inclusion of these mAbs in commercial catalogues would be of research and pharmaceutical interest.

Additional Information

For More Information About the Inventors

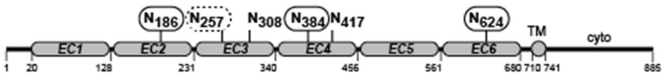
- Ann Palmenberg

Tech Fields

- Research Tools : Antibodies

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854

Figures



mAb name	Recognized CDHR3 EC domain	mAb isotype	Strongly reactive in western, IF, IP?	Comments
2H5*	EC1	IgG1,K	Y, Y, Y	
12A1	EC1	IgG2a,K	Y, Y, Y	
31C9*	EC2	IgG2a,K	Y, Y, Y	
28H12	EC2	IgG1,K	Y, Y, Y	
18D4*	EC5	IgG2A,K	**Y, Y, Y**	Strongest hybridoma titer, slightly stronger signal in all assays.
12E6	EC5	IgG1,K	Y, Y, Y	
13D5	EC5	IgG2b,K	Y, Y, Y	
9A1*	EC6	IgG1,K	Y, Y, Y	
20B8	EC6	IgG1,K	Y, Y, Y	