

# METHODS IN MOLECULAR BIOLOGY

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# Natural Antibodies

## Methods and Protocols

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## Preface

Natural antibodies, belonging to isotypes IgM, IgG, and IgA, were discovered nearly half a century ago. Natural antibodies are those that are detected in the serum of healthy organisms in the absence of pathology or intentional immunization. Most natural antibodies bind to one or more autoantigens and are called as natural autoantibodies. The importance of natural autoantibodies in immune regulation and in therapeutic application is now well established.

The role of natural antibodies in antigen presentation, pathogen elimination, cell survival and homeostasis, inflammation, cancer, and autoimmunity is exhaustively documented. While the basic properties of natural antibodies, origin, distribution, evolution in physiology and pathology, and functions were the subjects of thorough investigations, in parallel, a large body of highly interesting information has been generated on the methodology of isolation, identification, characterization, and quantification of natural antibodies in various situations. In this edition, we have gathered protocols from experts who have made significant contribution in this domain.

An overview of the progress in the understanding of the functions of natural antibodies is summarized by *Heinz Kohler*. Three chapters focus on the protocols for isolation of natural antibodies: while *Vogel and Horn* provide methods for purifying anti-FcεRIα autoantibodies from serum, *Schneider and colleagues* describe methods for separation of natural antibodies from human plasma, saliva, breast milk, and gastrointestinal fluid, and *Kolarova and colleagues* present protocols for purification of natural antibodies against tau protein. *Bannoudi and colleagues* detail protocols for unbiased RACE-based massive parallel surveys of human IgA antibody repertoires. In view of the role of natural antibodies in B cell survival and homeostasis, *Huo and colleagues* present protocols for the assessment of signaling events in B-1a cells, while *Mohr and Lino* narrate how microbiota influences the B1 and MZ B-cell numbers by the normal polyreactive immunoglobulins.

Certain intriguing functional properties of natural antibodies such as their anti-tumor cytotoxic activity (*Schwartz-Albiez and Dill*), hydrolysis and dissolution of their target antigens (*Meretoja and colleagues*), and the ability of natural antibodies to undergo enhanced polyreactivity (*Lecerf and colleagues*) are elaborated in subsequent chapters. Natural IgM antibodies recognizing oxidation-specific epitopes on circulating microvesicles (*Puhm and Binder*) and oxidized low-density lipoprotein and *Aggregatibacter actinomycetemcomitans* (*Wang and Hörkkö*) are discussed in the ensuing sections. Relevance of serological diagnosis of microbial antigens is discussed by *Jiménez-Munguía and colleagues* and detection of naturally occurring human antibodies against gangliosides is then narrated by *Hernández and Rodríguez-Zhurbenko*. Adenoviral vectors are the most widely used class of gene therapy vector in clinical trials. It is important to underline the role of natural antibodies in inhibiting viral vectors even in the absence of prior exposure to the virus. Thus, *Xu and colleagues* examine the protocols for evaluation of impact of natural IgM on adenovirus type 5 gene therapy vectors.

We are indebted to all the colleagues who shared time, energy, and experience for writing these protocols and to John Walker, the series editor, for his constant advice and support. We hope that the protocol series is highly helpful for laboratory approach to study the interesting features of natural antibodies.

*Paris, France*

*Srinivas V. Kaveri  
Jagadeesh Bayry*

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