

Development of Phospho Specific and Immunohistochemistry Grade Antibodies using a novel Rabbit Monoclonal Technology

by Maria Frolkis and Abe Couse

While mouse and rat monoclonal technologies have existed for several decades the emergence of a rabbit hybridoma technology has been desirable but elusive. Rabbits provide several specific advantages over mice for generating antibodies. Firstly rabbits are known to respond well to antigens which are not immunogenic in mice (1-2). For example peptides cannot generally be used in mice as they do not elicit an immune response. Secondly rabbit antibodies tend to be of very high affinity. Thirdly rabbit antibodies are ideal for use with rodent tissues.

The benefits of the rabbit immune system has been taken advantage of through the generation rabbit polyclonals for many years, but no technology existed for the creation of rabbit hybridomas from which rabbit monoclonals could be produced.

The availability of the first suitable fusion partner, created in 1995 by Dr. Katherine Knight, enabled the generation of stable rabbit hybridomas (3). The following year, Dr Robert Pytela and his colleagues further developed this cell line and established a robust system for the creation of rabbit monoclonal antibodies.

Using this proprietary rabbit monoclonal antibody (RabMAB) technology, Epitomics has developed a large number of antibodies which perform well in a variety of immunoassays (Fig.1). RabMAbs combine the advantage of high affinity, attributable to their rabbit origin with high specificity due to their monoclonal nature.

There are two specific areas in which our RabMab technology has proven to be especially useful. The first is in the generation of Phospho specific antibodies. Phospho-specific antibodies have emerged as key tools for studying signaling events that are associated with the pathogenesis of many diseases. These antibodies are developed to recognize and bind only to the phosphorylated form of a protein. We have begun to develop a series of phospho and other modification specific antibodies using this technology. A variety of techniques including western blot, dot blot, flow cytometry, immunohistochemical analysis and fluorescent immunostaining have been used by our group to functionally characterize the specificity of our RabMAbs (Fig.2-3).

The second area where RabMAbs have proven to be especially useful is immunohistochemistry. The high affinity and low background of RabMAbs makes them ideal affinity reagents for detection of antigens in formalin fixed paraffin embedded tissue sections. Epitomics has developed several high quality

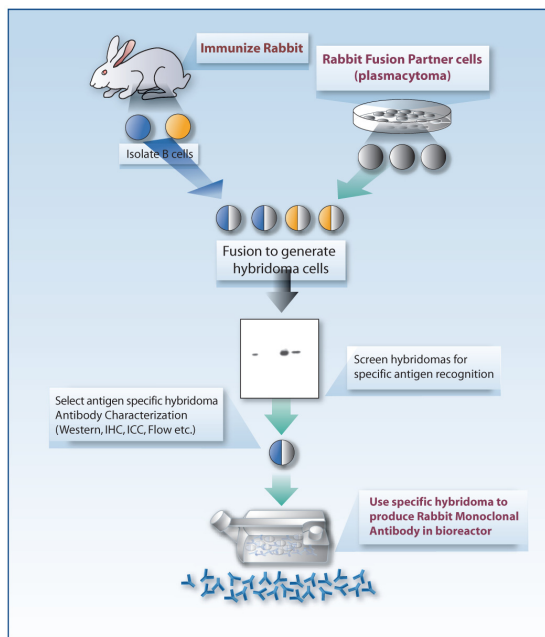


Figure 1. General procedure Epitomics uses for making rabbit monoclonals.

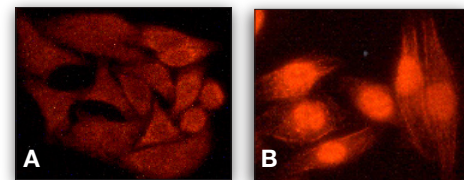


Figure 2. Immunofluorescent staining of HSP27 in (A) normal HeLa cells and (B) Hsp27 relocating to the nucleus upon heat shock using Phospho-HSP27 (pS82) RabMAB (clone E118).

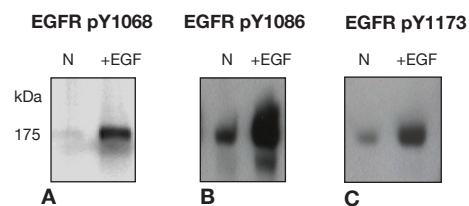


Figure 3. Phospho specific EGFR RabMAbs. Western blot analysis of A431 untreated cell lysates and A431+EGF treated cell lysates using the following : (A) Phospho-EGFR (pY1068) RabMAB (clone Y38) (B) Phospho-EGFR (pY1086) RabMAB (clone Y39) (C) Phospho-EGFR (pY1173) RabMAB (clone E124)

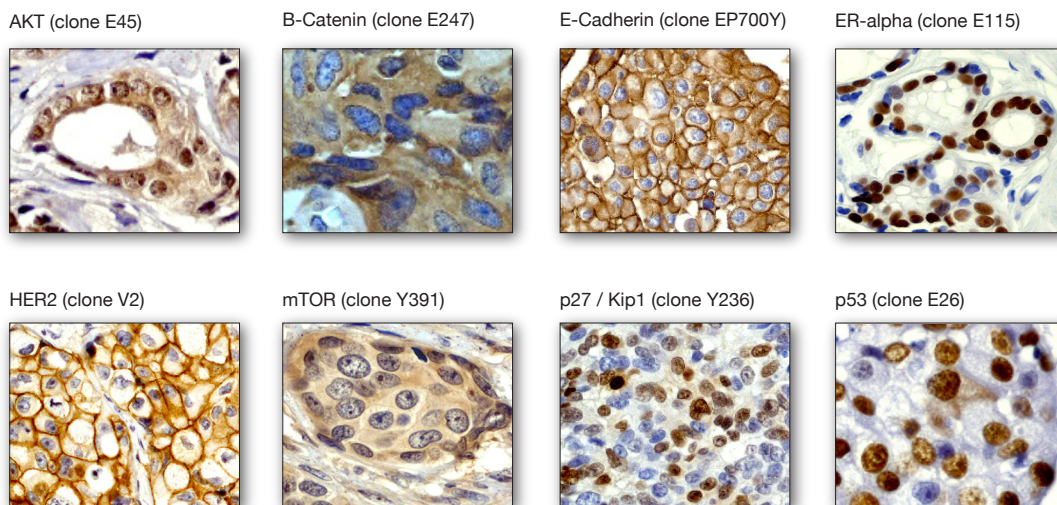


Figure 4. Immunohistochemical detection of breast cancer markers in paraffin embedded tissue sections, representing various stages of breast cancer, using Rabbit Monoclonal Antibodies.

IHC antibodies against various antigens which can be used in both basic research and clinical settings (Fig.4). Several studies conducted recently comparing RabMAbs to mouse monoclonals have found RabMAbs to be superior detection reagents (4-5). In addition to use in IHC rabbit monoclonals are proving to be excellent detection reagents for use in a variety of immunoassays.

References

1. Mage, R.G. et al., (2006) *Developmental and Comparative Immunology* **30**, 137-153.
2. Norrby, E. et al., (1987) *PNAS* **84**, 6572-576.
3. Spieker-Polet et al., (1995) *PNAS* **92**, 9348-524.
4. Rossi, S. et al., (2005) *Am J Clin Pathol* **124**, 0.
5. Ramos-Vara (2005) *Vet. Pathol.* **42**, 405-426.