

PrEST Antigens

Immunogens in the generation of
Triple A Polyclonals and PrecisA Monoclonals

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Atlas Antibodies now provide you with the PrEST Antigens in the product catalog as a complement to our highly validated Triple A Polyclonals and PrecisA Monoclonals.

Background

The antibodies in Atlas Antibodies' catalog are derived from recombinant human Protein Epitope Signature Tags (PrESTs). These recombinantly produced protein fragments consist of 50-150 amino acids, designed to have as low sequence identity as possible to other human proteins.

The protein fragments are expressed as fusion proteins with a dual tag consisting of a hexahistidyl (His₆) tag in frame with an immunopotentiating Albumin Binding Protein (ABP)-tag originating from Streptococcal Protein G.^{1,2}

Atlas Antibodies and the Human Protein Atlas project (HPA)^{3,4,5,6} use these PrEST Antigens for immunization. The resulting polyclonal antibodies are affinity purified against the corresponding PrEST Antigens in a three-step procedure to remove the tag-specific antibodies and to catch the PrEST-specific antibodies⁷.

PrEST Design

The 50-150 protein specific fragments are selected using a proprietary software to contain unique epitopes present in the native protein suitable for triggering the generation of antibodies of high specificity⁸. This is achieved by a complete human genome scanning to ensure that regions with the lowest homology to other human proteins are used as antigens for the generation of antibodies. In addition, signal peptides and membrane regions are avoided in the design.

The use of fragments of 50-150 amino acid residues facilitates cloning and protein expression and also provides conformational epitopes that could not be obtained using shorter peptides.

PrEST Antigen Production

For cloning of the PrESTs, a pool of RNA consisting of material from a number of human tissues is used in an RT-PCR approach. The amplified gene fragments are cloned and sequence verified. An *E. coli* recombinant protein expression system is used for expressing the clones and the products are purified using nickel-containing matrices (IMAC).

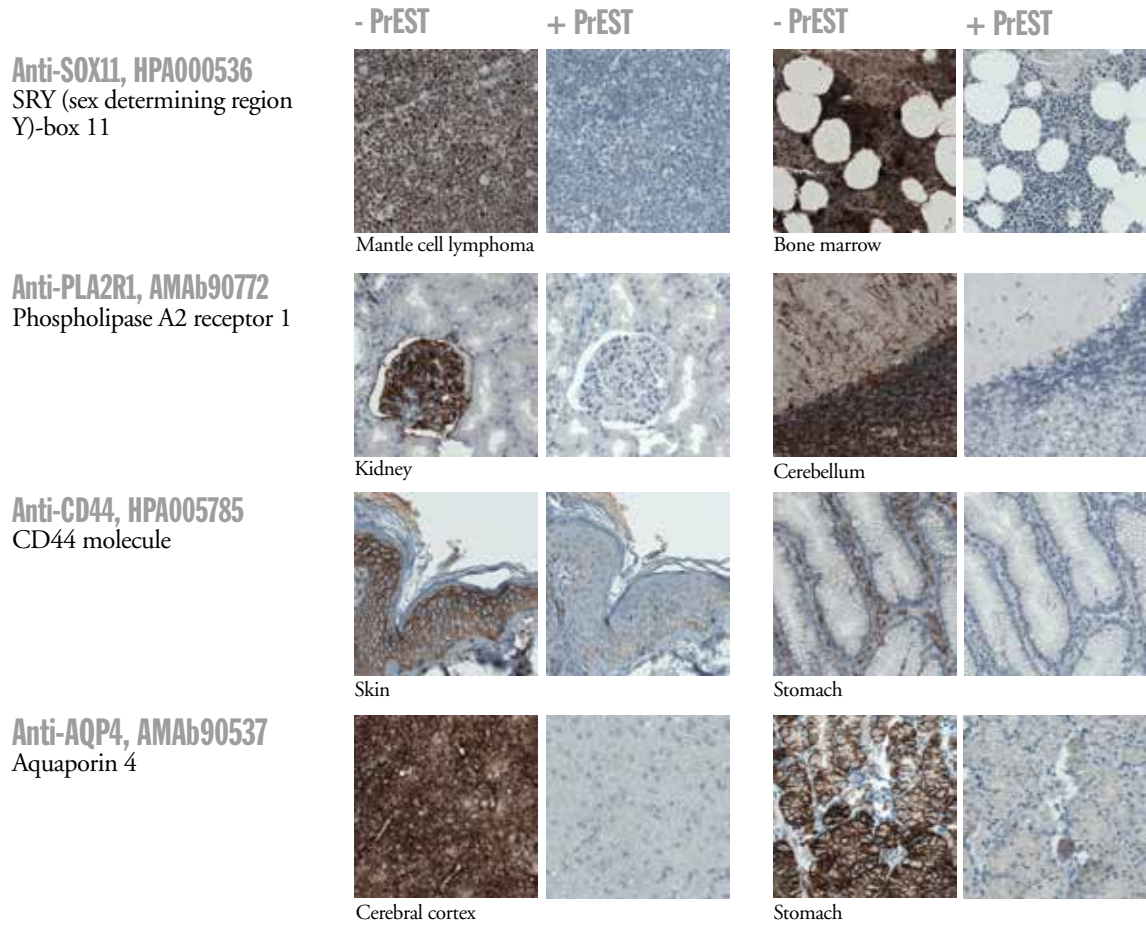
PrEST Antigen Validation and Quality Control

The expressed PrEST Antigens are validated using ESI mass spectrometry. Purity is analyzed using SDS page and the PrEST Antigen amount is being quantified using the Nanodrop system.



PrEST Antigens in pre-adsorption assays

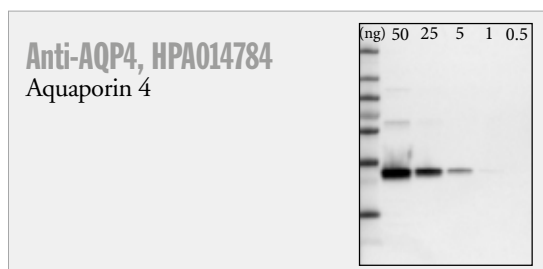
IHC staining results following pre-adsorption of antibody with corresponding PrEST Antigen



WB results following pre-adsorption of antibody with corresponding PrEST Antigen



PrEST Antigens as positive control in Western Blot



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You'll find we're Totally Human.