

Monospecific Mouse Monoclonal Antibodies

The immunohistochemical localization of specific antigens on formalin-fixed and paraffin embedded (FFPE) tissue sections plays key role in determining tumor tissue origin, prognostic marker expression and target protein expression. Traditionally, antibodies (monoclonal and polyclonal) are raised against specific proteins/peptides and screened by ELISA, gel electrophoresis and FFPE tissue stains. However, the antibody specificity has never been scrutinized by sophisticated method until recently.

Zeta's Monospecific Mouse Monoclonal Antibodies were produced through traditional hybridoma technologies. The clones were first screened by ELISA and gel electrophoresis to determine their specificity. The selected antibodies were further screened by staining FFPE sections. Only those antibodies that worked on FFPE sections were selected for protein array analysis.

A protein microarray(or protein chip) (Figure 1) for selection of monospecific antibody is a high-throughput method used to track the specific antigen-antibody interactions on a large scale. The chip consists of a support surface such as a glass slide, to which an array of capture proteins is bound. For monospecific antibody selection, close to 21,000 known human proteins are bound to a glass slide. Antibodies, labeled with a fluorescent dye, are added to the array.

Any interaction between the protein and antibody immobilizes protein emits a fluorescent signal that is read by a laser scanner. The intensity of fluorescent determines the specificity of an antibody. The top hits are plotted in X (target ranking)-Y (fluorescence intensity in standard deviations) axis (Figure 2). S-scores greater than 3 standard deviations over the next listed target are deemed statistically significant and indicate highly specific antibodies.

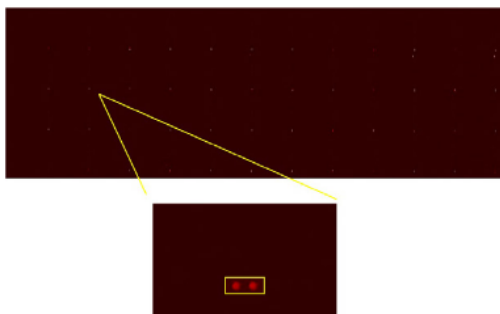


Figure 1: Microarray Analysis: Antibody cross-reactivity is evaluated using the CDI HuProt Human Proteome Microarray (~81% of the human proteome) and subsequently analyzed with GenePix Pro Image Acquisition and Analysis Software, the benchmark tool for the acquisition and analysis of microarray images. The top 3 "hits" are identified by cross-reference to the array map which stores the exact location of each protein. If the expected target is ranked #1 and the S-Score (the difference between Rank #1 and #2) is >3, then the antibody is considered monospecific.

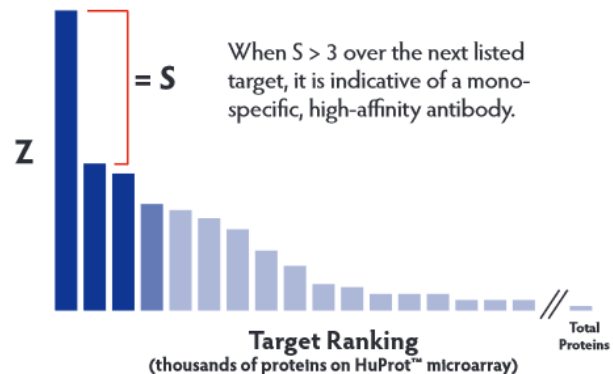


Figure 2: Statistical Analysis: Thousands of GenePix data points (from the microarray) are analyzed in terms of signal strength and ranked accordingly. The Z-score indicates the number of standard deviations above background seen for the mean signal bound by the target antigen. The S-score represents the difference between the Z-score of the target antigen and the next best hit on the array.

List of target-validated Monospecific monoclonal antibodies we offer (Sep 2020):

ALDH1A1 (ZM77)	Cyclin E (ZM121)	Nucleophosmin (ZM82)
Annexin A1 (ZM211)	Cytokeratin 20 (ZM42)	OCT-2 (ZM90)
BCL-6 (ZM22)	Cytokeratin 5 (ZM186)	p63 (ZM70)
Beta-catenin (ZM13)	E-cadherin (ZM63)	PAX-5 (ZM26)
BOB1 (ZM74)	EP-CAM (ZM131)	PAX-8 (ZM28)
Calretinin (ZM85)	Erythropoietin (ZM135)	Perforin (ZM159)
CD4 (ZM180)	Galectin-3 (ZM182)	PGP 9.5 (ZM160)
CD5 (ZM61)	GCDFP-15 (ZM23)	Podoplanin (ZM31)
CD8 (ZR216)	Kappa (ZM81)	PSAP (ZM162)
CD11c (ZM103)	Lysozyme (ZM120)	SOX-2 (ZM57)
CD19 (ZM179)	Mammaglobin (ZM193)	SOX-9 (ZM171)
CD20 (ZM86)	MBP (ZM202)	SOX-10 (ZM10)
CD21 (ZM75)	MBP (ZR109)	Surfactant (ZM124)
CD22 (ZM183)	Mesothelin (ZM25)	Synaptophysin (ZM208)
CD23 (ZM209)	MSH-2 (ZM210)	TdT (ZM51)
CD61 (ZM33)	MSH-6 (ZM99)	Thrombomodulin (ZM105)
CD123 (ZM80)	Napsin-A (ZM11)	TLE1 (ZM93)
CD163 (ZM29)	NGFR (ZM55)	TRAcP (ZM174)
Chromogranin A (ZM12)	NKX2.2 (ZM14)	Tryptase (ZM96)
Collagen IV (ZM177)	NKX3.1 (ZM95)	Ubiquitin (ZM191)
Cyclin D1 (ZM178)	NSE (ZM24)	ZAP-70 (ZM97)