

Humanized Monoclonal Antibodies for Immunoassay Development

☎ 1-631-624-4882 (USA) 44-161-818-6441 (Europe)

☎ 1-631-938-8221

✉ info@creative-diagnostics.com

📍 45-1 Ramsey Road, Shirley, NY 11967, USA

What is Humanized Monoclonal Antibodies?

Each variable region of the antibody contains three amino acid sequence hypervariable regions, which are binding sites of the antigen and are complementary to the structure of the antigenic determinant, and are referred to as antibody complementarity determining regions (CDRs). Amino acid sequences and spaces of the CDRs have polymorphism in the structure which is a factor determining the heterogeneity and affinity of antibodies. Other amino acids in the variable region serve as a backbone support moiety, called Framework Residue (FR), which is not in direct contact with the antigen. Amino acid sequence and spatial structure are relatively conservative, providing a skeleton for maintaining the typical three-dimensional structure of the antibody, indirectly affecting the antibody. The specificity and affinity of the antibody constant region and the antibody variable region FR are relatively conservative during the evolution process, and they have species specificity, which is the main factor causing the body to reject the reaction. Therefore, the basic principle of antibody humanization is to preserve the conserved sequence of the antibody as a human sequence, reduce the body rejection reaction, replace the antigen-binding region with the sequence of the antibody produced by the animal immunization, and maintain the specificity and affinity of the antibody.

Humanized antibodies are antibodies from non-human species whose protein sequences have been modified to increase their similarity to naturally occurring antibody variants in humans. The "humanization" process is typically applied to monoclonal antibodies (eg, antibodies developed as anticancer drugs) developed for human administration. Humanization may be necessary when the process of developing specific antibodies involves production in a non-human immune system, such as a mouse. The protein sequence of an antibody produced in this manner is partially different from a homologous antibody naturally present in humans and thus has potential immunogenicity when administered to a human patient. Humanized antibodies differ from chimeric antibodies. The latter also makes its protein sequence more similar to human antibodies, but carries a wider range of non-human proteins.

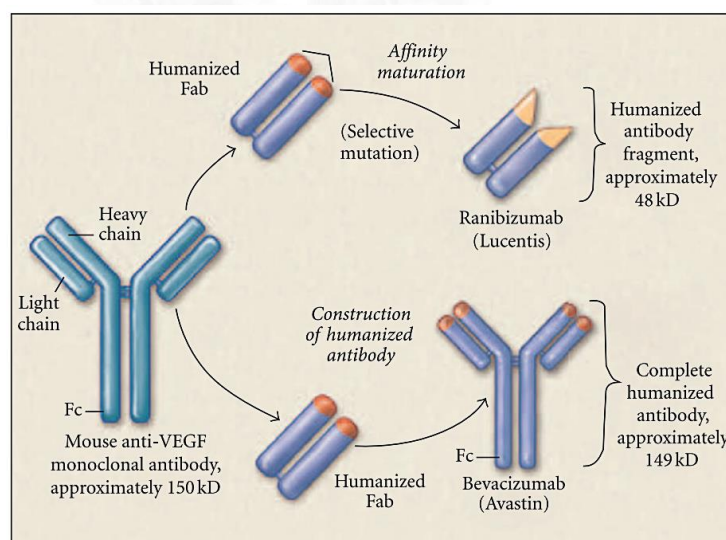


Figure 1. The process of Humanization of antibodies.

Creative Diagnostics now offers a newly developed set of humanized monoclonal antibodies (IgM, IgG or IgA) that can be used as calibrators, positive, negative or quality controls in assay development and manufacturing of diagnostic kits.

Product	Cat No.	Isotype	Research Area
Anti-Gliadin Chimeric Mab	CABT-L2430; CABT-L2431	IgA	Allergy and food intolerance
Anti-TGM2 Chimeric Mab	CABT-L2432; CABT-L2433	IgA	Allergy and food intolerance
Anti- β -lactoglobulin Chimeric Mab	CABT-L2429	IgA	Allergy and food intolerance
Anti-Ovalbumin Chimeric Mab	CABT-L2434; CABT-L2435	IgA; IgE	Allergy and food intolerance
Anti-Peanut antigen Chimeric Mab	CABT-L2436; CABT-L2437	IgA; IgE	Allergy and food intolerance
Anti-Penicillin Chimeric Mab	CABT-L2440; CABT-L2441	IgE	Allergy and food intolerance
Anti-Wasp venom Chimeric Mab	CABT-L2438; CABT-L2439	IgE	Allergy and food intolerance
Anti-LKM-1 Chimeric Mab	CABT-L2414	IgG	Auto-immunity
Anti-Human SRP54 Chimeric Mab	CABT-L2415	IgG	Auto-immunity
Anti-CMV glycoprotein B Chimeric Mab	CABT-L2443; CABT-L2444	IgG; IgM	Infectious disease
Anti-CMV HHV5 Chimeric Mab	CABT-L2442	IgA	Infectious disease
Anti-Cytomegalovirus Chimeric Mab	CABT-L2394; CABT-L2395	IgM	Infectious disease
Anti-IAV H1N1 Chimeric Mab	CABT-L2446	IgA	Infectious disease
Anti-IAV H3N2 Chimeric Mab	CABT-L2447	IgA	Infectious disease
Anti-RSV F protein Chimeric Mab	CABT-L2445	IgA	Infectious disease
Anti-Measles Chimeric Mab	CABT-L2396	IgM	Infectious disease
Anti-Mumps Chimeric Mab	CABT-L2397	IgM	Infectious disease
Anti-Rubella Chimeric Mab	CABT-L2399	IgM	Infectious disease
Anti-T. gondii Chimeric Mab	CABT-L2401	IgM	Infectious disease
Anti-VZV Chimeric Mab	CABT-L2402	IgM	Infectious disease
Anti-IgE Chimeric Mab	CABT-L2425; CABT-L2426	IgA; IgG	Other
Anti-Secretory component Chimeric Mab	CABT-L2427; CABT-L2428	IgG	Other

Why Choose Humanized Monoclonal Antibodies?

Humanized mouse monoclonal antibodies are mouse/human chimeric monoclonal antibodies produced in transgenic mice by replacing the mouse sequence of the heavy chain constant region (IgM, IgG or IgA loci) by the corresponding human sequence. After immunization with the antigen of interest, generated antibody clones are cultivated by standard hybridoma techniques. They consist of the human constant region of the heavy chain, mouse variable region of the heavy chain and mouse light chain. The human constant region of the heavy chain can be directly recognized by the anti-human conjugate, which is used in numerous in vitro diagnostic assays. Due to the following advantages, humanized antibodies have become better Calibrators and Controls in the development of kits.

✧ High affinity and specificity

✧ Minimal lot to lot variation

✧ Industrial batch sizes

✧ Continuous availability

✧ Constant quality

✧ Avoid heterophilic interference

How to Verify Humanized Antibodies?

Antibody validation is defined in the following points: proving specificity (the ability of an antibody to differentiate between different antigens), proving specificity in the application in which it is going to be used, proving affinity (the strength with which an antibody binds an epitope) and finally proving reproducibility.

● Antibody Specificity

Specificity measures the degree to which an antibody differentiates between different antigens. It is possible for an antibody to be sensitive for a protein of interest but still cross-react with other proteins and therefore lack specificity. In this sense, the confirmation that an antibody binds specifically to the intended protein is its minimal requirement and the first thing to look up to in the antibodies verification.

Specificity measures the extent to which antibodies differentiate between different antigens. Antibodies may be sensitive to proteins of interest, but still cross-react with other proteins and therefore lack specificity. In this sense, the confirmation of specific binding of an antibody to a target protein is its minimum requirement. WB is the first step in assessing the specificity of new antibodies. Besides, immunoprecipitation and Chromatin Immunoprecipitation can be also used for specificity measures.

Cat: CABT-L2446

Chimeric Mab (IgA) directed against IAV H1N1

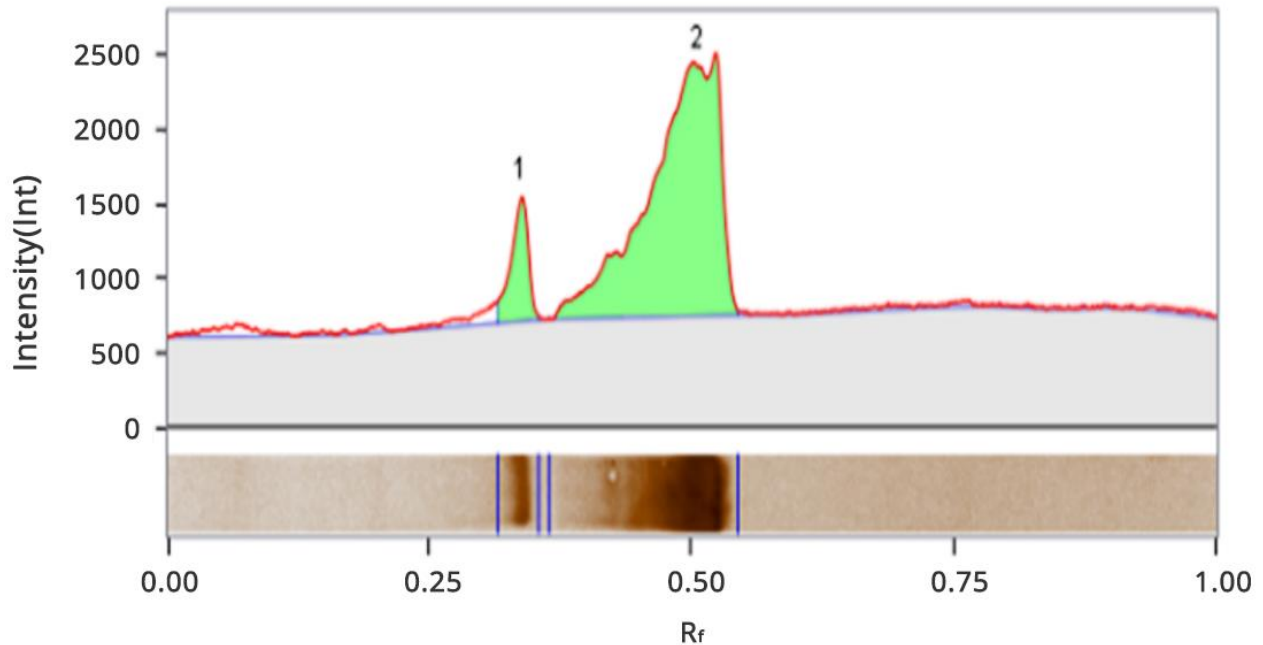


Figure 2. Detection of IAV (H1N1) by WB with Os9TB6.

● Antibody Affinity

Antibody binding affinity is another parameter that can be used for antibody validation. It refers to the strength with which an antibody molecule binds an epitope. It is typically reported by the equilibrium dissociation constant (KD), which is the ratio of the antibody dissociation rate or koff (how quickly it dissociates from its antigen), to the antibody association rate or kon of the antibody (how fast it binds to its antigen). For determining antibody affinity, several methods have been used. Among them are methods based on ELISA, and other biophysical methods such as microscale thermophoresis (MST) and surface plasmon resonance (SPR).

ELISA-based Methods

ELISA methods are the most popular methods for studying antibody affinities. They do not require considerable quantities of antibodies and antigens nor their purification. In this method, a fix antibody concentration is incubated with antigen in solution until steady state is reached. Then, the concentration of unbound antibody is measured by indirect ELISA. This method need previous setup experiments though, to ascertain that the antibody concentration used is in the linear range of the ELISA response (so that the absorbance is proportional to the Ab concentration) and that only a small fraction of the total free antibody in solution is retained on the plate (so that the measurement does not significantly affect the equilibrium in solution).

Cat: CABT-L2446

Chimeric Mab (IgA) directed against IAV H1N1

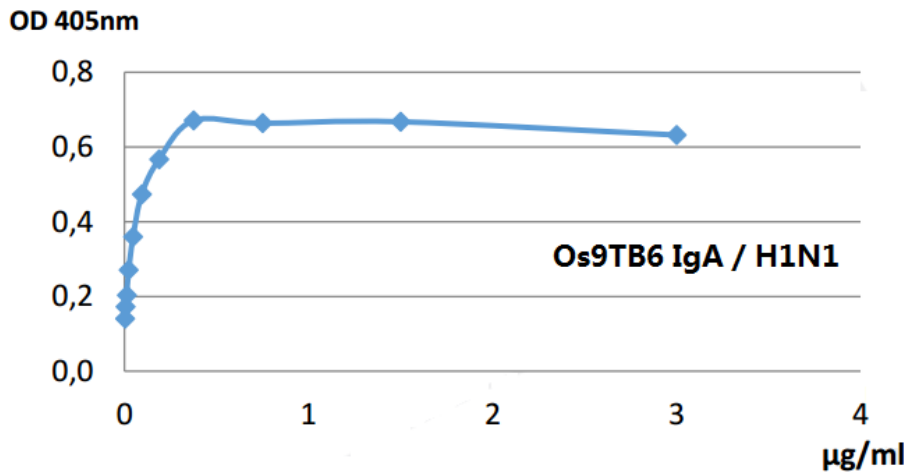


Figure 3. Os9TB6 IgA1 reactivity in ELISA on 100 ng of IAV.

Cat: CABT-L2450

Chimeric Mab (IgM) directed against IAV H1N1/H3N2

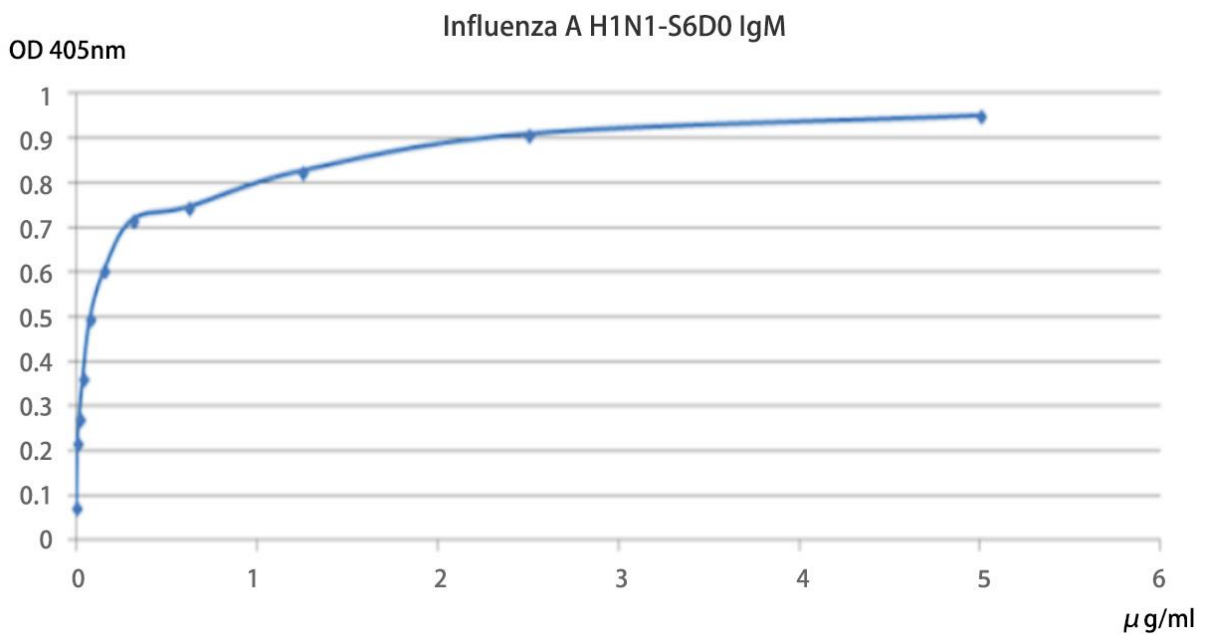


Figure 4. S6D0 IgM reactivity in ELISA on 100 ng of IAV.

MST and SPR

MST is a biophysical method that can access molecular affinities in a wide concentration range. It measures the motion of molecules along microscopic temperature gradients (thermophoresis) induced by an infrared laser. SPR is an optical technique utilized for detecting molecular interactions, where one of the molecules is mobile, and the other is immobilized in a metal film. Binding of these molecules changes the refractive index of the film. Thus, when polarized light impact upon the film, the angle of extinction of light is altered which can be monitored by an optical detector.


● Antibody Reproducibility

An essential criterion for validation is antibody reproducibility. That is, the usage of the same antibody over time, even with different lots, would yield similar results. Thus, it is crucial for researchers to always test the reproducibility of antibodies. This gains even more importance when using polyclonal antibodies, since often same catalog number from a supplier may mean different antibodies.



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