

For the V_L domain the humanized variant LC1 was ranked best from the T-cell epitope screen. Following the alignment humanized variants LC3, LC4 and LC5 all were the most homologous to the murine light chain.

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Screening for post-translational modifications:

Fv Glycosylation

The N-linked glycosylation motif is NXS/T where X is any amino acid except proline. This motif is not present in the murine or humanized variants of VII/20 mAb V_H. The motif NQS is present in the framework 1 region of the murine variant LC0. This motif was removed during the humanization process and is not present in any of the humanized light chain variants.

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Deamidation

The amino acid motifs SNG, ENN, LNG, and LNN can be prone to deamidation of asparagines to aspartic acid (Chelius et al, *Anal Chem* 77(18): 6004-11, 2005). Asparagine within other motifs is less prone to deamidation. None of these four motifs are present in the murine or humanized variants derived from VII/20 mAb V_H or V_L.

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The foregoing data demonstrate the generation of twenty-five humanized variants (marked in the following text as CA9hu-1_HCxLCx) having humanized variable domains and human Ig constant domains.

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Example 2: Humanized antibodies derived from IV/18 mAb

This example demonstrates the construction and characterization of humanized antibody variants CA9hu-2.

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The humanization process utilized a combination of standard CDR-grafting technologies coupled with the latest research on antibody structure and up-to-date database of mature human IgG sequences. Firstly, the IV/18 murine antibody variable domains were sequenced. The Complementarity Determining Regions (CDRs) were identified using the Immunogenetics Information System® (IMGT®) or the Kabat numbering system. For optimal retention of CDR-loop conformation, both numbering systems were used to identify CDRs within murine variable heavy (V_H) as well as variable light (V_L) domains. Subsequently, a number of human framework sequences were identified and used as “acceptor” frameworks (in the text below) for the CDR sequences. Each of the V_H domains was synthesized in-frame with a human IgG isotype constant domain sequence (allotype G1m17,1).

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