in the amino acid sequence (4) described above are deleted, substituted or added can be exemplified.

Incidentally, the term "several" as used herein refers to 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 or 2.

As the amino acid substitution in this specification, a conservative amino acid substitution is preferred. The conservative amino acid substitution refers to a substitution occurring within a group of amino acids related to amino acid side chains. Preferred amino acid groups are as follows: an acidic group (aspartic acid and glutamic acid); a basic group (lysine, arginine, and histidine); a non-polar group (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan); and an uncharged polar family (glycine, asparagine, glutamine, cysteine, serine, threonine, and tyrosine). More preferred amino acid groups are as follows: an aliphatic hydroxy group (serine and threonine); an amide-containing group (asparagine and glutamine); an aliphatic group (alanine, valine, leucine, and isoleucine); and an aromatic group (phenylalanine, tryptophan, and tyrosine). Such an amino acid substitution is preferably performed within a group which does not impair the properties of a substance having the original amino acid sequence.

[0113]

As an antibody which has a preferred combination of a heavy chain and a light

of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid residues 20 to 141 of SEQ ID NO: 91 and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid residues 21 to 128 of SEQ ID NO: 75; and an antibody consisting of a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid residues 20 to 141 of SEQ ID NO: 91 and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid residues 21 to 128 of SEQ ID NO: 77 can be exemplified.

As an antibody which has a more preferred combination of a heavy chain and a light chain described above,

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 71;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 73;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in

SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 75;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 77;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 79;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 81;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 85 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 83;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in

SEQ ID NO: 91 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 71;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 91 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 73;

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 91 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 75; and

an antibody consisting of a heavy chain consisting of an amino acid sequence represented by amino acid numbers 20 to 471 in SEQ ID NO: 91 and a light chain consisting of an amino acid sequence represented by amino acid numbers 21 to 233 in SEQ ID NO: 77.

[0114]

Furthermore, as an antibody which has a preferred combination of a heavy chain and a light chain described above, ~~As a more preferred combination,~~ an antibody consisting of a heavy chain comprising an amino acid sequence of SEQ ID NO: 85 and a light chain comprising an amino acid sequence of SEQ ID NO: 71; an antibody consisting of a heavy chain

comprising an amino acid sequence of SEQ ID NO: 85 and a light chain comprising an amino acid sequence of SEQ ID NO: 73; an antibody consisting of a heavy chain comprising an amino acid sequence of SEQ ID NO: 85 and a light chain comprising an amino acid sequence of SEQ ID NO: 75; an antibody consisting of a heavy chain comprising an amino acid sequence of SEQ ID NO: 85 and a light chain comprising an amino acid sequence of SEQ ID NO: 77; an antibody consisting of a heavy chain comprising an amino acid sequence of SEQ ID NO: 85 and a light chain

the heavy chain of the mouse antibody M30 is represented by SEQ ID NO: 50 in the Sequence Listing, and the amino acid sequence thereof is represented by SEQ ID NO: 51. Further, the sequences of SEQ ID NOS: 50 and 51 are shown in Fig. 21.

The determined nucleotide sequence of the cDNA encoding the light chain of the mouse antibody M30 is represented by SEQ ID NO: 52 in the Sequence Listing, and the amino acid sequence thereof is represented by SEQ ID NO: 53 in the Sequence Listing. The sequences of SEQ ID NOS: 52 and 53 are shown in Fig. 22.

Further, the amino acid sequences of the heavy chain and the light chain were analyzed by comparison using KabatMan (see PROTEINS: Structure, Function and Genetics, 25 (1996), 130-133), which is the database of the amino acid sequences of antibodies. As a result, it was found that in the heavy chain of the mouse antibody M30, the amino acid sequence represented by amino acid numbers 20 to 141 in SEQ ID NO: 51 in the Sequence Listing is the variable region. It was also found that in the light chain of the mouse antibody M30, the amino acid sequence represented by amino acid numbers 23 to 130 in SEQ ID NO: 53 in the Sequence Listing is the variable region.

Example 7. Production of chimeric antibody M30 (cM30 antibody)

7)-1 ~~Production of universal expression vectors pEF6KCL and~~

~~pEF1FCCU~~

~~7)-1-1~~ Construction of chimeric and humanized light chain expression vector pEF6KCL

By performing PCR using a plasmid pEF6/V5-HisB (Invitrogen Corporation) as a template and also using the following primers, a DNA fragment from immediately downstream of BGHpA (Sequence Position: 2174) to SmaI (Sequence Position: 2958) (a DNA fragment comprising f1 origin of replication and SV40 promoter and origin, hereinafter referred to as "fragment A") was obtained.

Primer 20

5'-ccacgcgccctgtagcggcgcattaagc-3' (SEQ ID NO: 54 in the Sequence Listing)

Primer 21

5'-aaacccgggagctttttgcaaaagcctagg-3' (SEQ ID NO: 55 in the Sequence Listing)

The obtained fragment A and a DNA fragment (SEQ ID NO: 56, hereinafter referred to as "fragment B", the sequence of SEQ ID NO: 56 is also shown in Fig. 23) comprising a DNA sequence encoding a human κ chain secretory signal, a human κ chain constant region, and a human poly-A additional signal were ligated to each other by overlap extension PCR. The thus obtained DNA fragment in which the fragment A and the fragment B were ligated to each other was digested with the restriction enzymes KpnI and SmaI, which was ligated to a plasmid

sequences in the Kabat Database (Nuc. Acid Res. 29, 205-206 (2001)) of antibody amino acid sequences. As a result, a mAb49'CL antibody (GenBank in NCBI: D16838.1 and D16837.1) was selected as an acceptor based on a sequence homology of 70% in the framework region.

The amino acid residues in the framework region of mAb49'CL were aligned with the amino acid residues of M30, and the positions where different amino acids were used were identified. The positions of these residues were analyzed using the three-dimensional model of M30 constructed above. Then, donor residues to be grafted onto the acceptor were selected according to the criteria provided by Queen et al. (Proc. Natl. Acad. Sci. USA 86, 10029-10033 (1989)). By transferring some selected donor residues to the acceptor antibody, humanized M30 antibody sequences were constructed as described in the following Example.

9)-2 Humanization of M30 heavy chain

9)-2-1 M30-H1-type heavy chain:

A humanized M30 heavy chain designed by substituting amino acid numbers 20 (glutamic acid), 24 (glutamine), 28 (proline), 30 (leucine), 31 (valine), 35 (alanine), 39 (methionine), 57 (lysine), 59 (lysine), 67 (isoleucine), 86 (lysine), 87 (alanine), 89 (glutamine), 91 (serine), 93 (lysine), 95 (serine), 106 (threonine), 110 (serine), 136