

Claims

- [Claim 1] An insulin analog peptide comprising an A-chain of SEQ ID NO: 3 indicated in the following General Formula 1 and a B-chain of SEQ ID NO: 4 indicated in the following General Formula 2:
(General Formula 1)
Xaa1-Ile-Val-Glu-**Xaa2**-Cys-Cys-Thr-Ser-Ile-Cys-**Xaa3**-Leu-**Xaa4**-Gln-**Xaa5**-Glu-Asn-**Xaa6**-Cys-**Xaa7**(SEQ ID NO: 3)
wherein
Xaa1 is alanine, glycine, glutamine, histidine, glutamic acid, or asparagine;
Xaa2 is alanine, glutamic acid, glutamine, histidine, or asparagine;
Xaa3 is alanine, serine, glutamine, glutamic acid, histidine, or asparagine;
Xaa4 is alanine, tyrosine, glutamic acid, histidine, lysine, aspartic acid, or asparagine;
Xaa5 is alanine, leucine, tyrosine, histidine, glutamic acid, or asparagine;
Xaa6 is alanine, tyrosine, serine, glutamic acid, histidine, or asparagine;
and
Xaa7 is asparagine, glycine, histidine, or alanine; and
(General Formula 2)
Phe-Val-Asn-Gln-His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-**Xaa8**-Leu-Val-Cys-Gly-Glu-Arg-Gly-Phe-**Xaa9**-Tyr-**Xaa10**-**Xaa11**-Lys-Thr (SEQ ID NO: 4)
wherein
Xaa8 is tyrosine, glutamic acid, or aspartic acid, or is absent;
Xaa9 is phenylalanine, or is absent;
Xaa10 is threonine, or is absent; and
Xaa11 is proline, glutamic acid, or aspartic acid, or is absent;
(with the proviso that the peptide comprising the A-chain of SEQ ID NO: 1 and the B-chain of SEQ ID NO: 2 is excluded).
- [Claim 2] The insulin analog peptide of claim 1, wherein, in the B-chain of SEQ ID NO: 4, Xaa8 is tyrosine, Xaa9 is absent, and Xaa10 is threonine.
- [Claim 3] The insulin analog peptide of claim 1, wherein, in the B-chain of SEQ ID NO: 4, Xaa8 is tyrosine, Xaa9 is phenylalanine, and Xaa10 is absent.
- [Claim 4] The insulin analog peptide of claim 1,

- wherein, in the A-chain of SEQ ID NO: 3, Xaa1 is glycine, Xaa2 is glutamine, Xaa3 is serine, Xaa4 is glutamic acid, Xaa5 is leucine, Xaa6 is tyrosine, and Xaa7 is asparagine, and
in the B-chain of SEQ ID NO: 4, Xaa8 is tyrosine, Xaa9 is phenylalanine, Xaa10 is threonine, and Xaa11 is proline.
- [Claim 5] The insulin analog peptide of claim 4, wherein the peptide comprises an amino acid sequence represented by SEQ ID NO: 16.
- [Claim 6] The insulin analog peptide of claim 1,
wherein, in the A-chain of SEQ ID NO: 3, Xaa1 is glycine, Xaa2 is glutamine, Xaa3 is serine, Xaa4 is asparagine, Xaa5 is leucine, Xaa6 is tyrosine, and Xaa7 is asparagine, and
in the B-chain of SEQ ID NO: 4, Xaa8 is tyrosine, Xaa9 is phenylalanine, Xaa10 is threonine, and Xaa11 is proline.
- [Claim 7] The insulin analog peptide of claim 6, wherein the peptide comprises an amino acid sequence represented by SEQ ID NO: 18.
- [Claim 8] The insulin analog peptide of claim 1,
wherein, in the A-chain of SEQ ID NO: 3, Xaa1 is glycine, Xaa2 is glutamine, Xaa3 is serine, Xaa4 is glutamic acid, Xaa5 is leucine, Xaa6 is tyrosine, and Xaa7 is asparagine, and
in the B-chain of SEQ ID NO: 4, Xaa8 is tyrosine, Xaa9 is absent, Xaa10 is threonine, and Xaa11 is proline.
- [Claim 9] The insulin analog peptide of claim 8, wherein the peptide comprises an amino acid sequence represented by SEQ ID NO: 20.
- [Claim 10] The insulin analog peptide of claim 1, wherein, in the A-chain of SEQ ID NO: 3, Xaa1 is glycine, Xaa2 is glutamine, Xaa3 is serine, Xaa4 is alanine, Xaa5 is leucine, Xaa6 is tyrosine, and Xaa7 is asparagine, and
in the B-chain of SEQ ID NO: 4, Xaa8 is glutamic acid, Xaa9 is absent, Xaa10 is threonine, and Xaa11 is proline.
- [Claim 11] The insulin analog peptide of claim 10, wherein the peptide comprises an amino acid sequence represented by SEQ ID NO: 22.
- [Claim 12] A nucleic acid encoding the insulin analog peptide according to any of claims 1 to 11.
- [Claim 13] The nucleic acid of claim 12, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 15, 17, 19, and 21.
- [Claim 14] A recombinant expression vector comprising the nucleic acid according to claim 12.
- [Claim 15] A transformant which is transformed with the recombinant expression

- vector according to claim 14.
- [Claim 16] The transformant of claim 15, wherein the transformant is *E. coli*.
- [Claim 17] A method of preparing the insulin analog peptide according to claim 1, comprising:
- a) preparing a recombinant expression vector comprising a nucleic acid encoding the insulin analog peptide according to claim 1;
 - b) transforming the recombinant expression vector into a host cell and obtaining a transformant therefrom;
 - c) culturing the transformant and expressing the insulin analog peptide; and
 - d) isolating and purifying the expressed insulin analog peptide.
- [Claim 18] The method of claim 17, wherein the nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 15, 17, 19, and 21.
- [Claim 19] The method of claim 17, wherein the transformant is *E. coli*.
- [Claim 20] The method of claim 17, wherein the isolating and purifying comprise:
- d-1) obtaining the transformant cells from the culture and pulverizing the same;
 - d-2) recovering the expressed insulin analog peptide from the pulverized cell lysate followed by refolding the same;
 - d-3) purifying the refolded insulin analog peptide by cation exchange chromatography;
 - d-4) treating the purified insulin analog peptide with trypsin and carboxypeptidase B; and
 - d-5) sequentially purifying the treated insulin analog peptide by cation exchange chromatography and anion exchange chromatography.
- [Claim 21] A pharmaceutical composition for treating diabetes comprising the insulin analog peptide according to any of claims 1 to 11 and a pharmaceutically acceptable carrier.