

WHAT IS CLAIMED

- 1) An isolated FN3 domain that specifically binds to a human CD8A protein comprising the amino acid sequence of SEQ ID NO: 35.
- 2) The isolated FN3 domain of claim 1, wherein the FN3 domain cross-reacts with cynomolgus monkey CD8A protein comprising the amino acid sequence of SEQ ID NO: 271.
- 3) The isolated FN3 domain of claim 2, wherein
 - a) the FN3 domain is based on a Tencon sequence of SEQ ID NO: 1;
 - b) the FN3 domain is based on a Tencon27 sequence of SEQ ID NO: 4; and/or
 - c) the FN3 domain is isolated from the library comprising the sequence of SEQ ID NOs: 2, 3, 5, 6, 7 or 8.
- 4) The isolated FN3 domain of claim 3, wherein the FN3 domain is conjugated to a second molecule.
- 5) The isolated FN3 domain of claim 4, wherein the second molecule is a detectable label.
- 6) The isolated FN3 domain of claim 5, wherein the detectable label is a radioactive isotope, magnetic beads, metallic beads, colloidal particles, a fluorescent dye, an electron-dense reagent, an enzyme, biotin, digoxigenin, or hapten.
- 7) The isolated FN3 domain of claim 3, wherein the FN3 domain has a cysteine substitution at residue position 54 corresponding to SEQ ID NOs 79, 81, 83, 89, 122 and 68.
- 8) The isolated FN3 domain of claim 3, wherein the FN3 domain does not activate CD8⁺ T-cells *in vitro* as measured by the enzyme-linked immunospot (ELISPOT) assay.
- 9) The isolated FN3 domain of claim 3, wherein the FN3 domain competes for binding to human CD8A protein with the FN3 domain comprising the amino acid sequence of one of SEQ ID NOs: 229-234.
- 10) The isolated FN3 domain of claim 3, wherein the FN3 domain binds to human CD8A protein with an affinity (K_D) of between about 0.02 to about 6.6 nM as measured by surface plasmon resonance performed according to the conditions described in Example 3.

- 11) The isolated FN3 domain of claim 3, wherein the FN3 domain comprises any one of the amino acid sequences of SEQ ID NOs: 40-269.
- 12) The isolated FN3 domain of claim 11, further comprising a methionine at the N-terminus of the FN3 domain.
- 13) The isolated FN3 domain of claim 3, wherein the FN3 domain is coupled to a half-life extending moiety.
- 14) The isolated FN3 domain of claim 13, wherein the half-life extending moiety is a CD8 binding molecule, albumin, an albumin variant, an albumin-binding molecule, a polyethylene glycol (PEG), CD8, CD8 variant, or at least a portion of an Fc region of an immunoglobulin.
- 15) An isolated polynucleotide encoding the FN3 domain of claim 11.
- 16) A vector comprising the polynucleotide of claim 15.
- 17) An isolated host cell comprising the vector of claim 16.
- 18) A method of producing an FN3 domain that specifically binds to a human CD8A protein, comprising culturing the isolated host cell of claim 17 under conditions such that the FN3 domain is expressed, and purifying the FN3 domain.
- 19) A diagnostic kit comprising the FN3 domain of claim 11.
- 20) A diagnostic or capture agent comprising the FN3 domain of claim 11.
- 21) The diagnostic or capture agent of claim 20 wherein the FN3 domain has a cysteine substitution at residue position 54 corresponding to SEQ ID NOs 79, 81, 83, 89, 122 and/or 68.
- 22) The diagnostic agent of claim 21 wherein the substituted cysteine is conjugated to Zr-89 or I-124.
- 23) A method of detecting CD8-expressing cells in a biological sample comprising treating the biological sample with the diagnostic reagent of claim 20 and evaluating the binding of the biological sample to the FN3 domain of such diagnostic agent.
- 24) The method of claim 23 wherein the diagnostic agent has a cysteine substitution at residue position 54 of SEQ ID NOs 79, 81, 83, 89, 122 and/or 68 and the substituted cysteine is conjugated to Zr-89 or I-124.