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(71) Applicant: **IMMUNOCORE LIMITED** [GB/GB]; 92
PARK DRIVE, MILTON PARK, ABINGDON, OXON
OX14 4RY (GB).

(72) Inventors: **PATEL, Viren**; c/o Immunocore Limited, 92
Park Drive, Milton Park, Abingdon, Oxon OX14 4RY (GB).
JOSEPH, Erin; c/o Immunocore Limited, 92 Park Drive,
Milton Park, Abingdon, Oxon OX14 4RY (GB).

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(54) Title: METHOD FOR PURIFYING SMALL MULTI-DOMAIN PROTEINS

(57) Abstract: The present disclosure provides a method for purifying a T cell engaging peptide-major histocompatibility complex binding domain (TCE-pMHC) molecule produced by a host cell, the method comprising: isolating a supernatant of a host cell culture comprising the TCE-pMHC molecule to form Product (a), subjecting Product (a) to affinity chromatography wherein the TCE-pMHC molecule is eluted from the affinity chromatography at a pH of about 3.0 to about 4.0 to form Product (b); neutralizing Product (b) by adding MES buffer with a pH of less than 7.0 to form Product (c), wherein Product (c) has a pH of about 5.0 to about 7.0; and subjecting Product (c) to anion exchange chromatography to form Product (d), wherein the pMHC binding domain and the T cell engaging immune effector domain are capable of binding to a pMHC complex and a T cell respectively.



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