Claims

1. A two-part device, wherein a first part is a multicomponent **TCR ORF reconstitution and engineering system (TORES)**, and a second part is a multicomponent **engineered TCR-presenting cell system (eTPCS)**, wherein the TORES comprises two separate components, wherein the first component **1A** is a vector carrying variable and constant (V-C) T-cell receptor (TCR) gene segments, and the second component **1B** is a vector carrying joining (J) TCR gene segments, and wherein operation of **a TORES** provides one or more genetic integration vectors, components 2C and/or 2E, each encoding an analyte TCR ORF selected from

    a. a native TCR chain

    b. a sequence-diversified TCR chain

    c. a synthetic TCR chain

    and wherein the eTPCS comprises a first component **eTPC,** designated **component 2A,** wherein component 2**A**

    a. Lacks endogenous expression of TCR chains alpha, beta, delta and gamma, and

    b. Expresses CD3 proteins which are conditionally presented on the surface of the cell only when the cell expresses a complementary pair of TCR chains and

    c. Contains further components **designated 2B and 2D**, genomic receiver sites, each for integration of a single ORF encoding one analyte TCR chain of alpha, beta, delta or gamma,

    and wherein the genomic receiver sites 2B and 2D are each selected from

        a. A synthetic construct designed for recombinase mediated cassette exchange (RMCE)

        b. A synthetic construct designed for site directed homologous recombination;

    and wherein **component 2C and 2E**, are matched to component 2**B and 2D, respectively**, and wherein the component 2**C and 2E** are designed to deliver a single ORF encoding one analyte TCR chain of alpha, beta, delta and/or gamma, and

wherein 2C and/or 2E optionally encodes a selection marker of integration, such that the analyte TCR chains can be expressed as TCR surface protein in complex with the CD3 (**TCRsp**) on component 2**A.**

2. A two-part device according to claim 1, wherein the one or more genetic integration vectors, components 2C and/or 2E, are used as input in the second part of the two-part device.

3. A two-part device according to **claim 1,** wherein **eTPCS** provides one or more analyte eTPC in which one or more TORES-derived analyte TCR chains are presented, and the one or more analyte eTPC is selected from

    a. An eTPC expressing a TCR pair (eTPC-t) and/or

    b. An eTPC expressing one TCR chain (eTPC-x) and/or

    c. one or more libraries thereof.

4. A two-part device according to claim 3, wherein a pair of analyte TCR chains are expressed as TCR surface proteins in complex with CD3 (**TCRsp**) by an analyte eTPC.

5. A two-part device according to claim 1, wherein component **1A** is a V-C entry vector containing
    a. origin of replication,
    b. a first positive selection marker,
    c. 5’ genetic element, or elements,
    d. Kozak Sequence,
    e. TCR variable gene segment,
    f. a first Type IIS sequence, for site specific recognition and cleavage by a Type IIS restriction enzyme,
    g. a negative selection marker,
    h. a second Type IIS sequence,
    i. TCR constant gene segment, and
    j. 3’ genetic element, or elements.

6. A two-part device according to claim 1 or 5, wherein component **1B** is a J donor vector containing

a. origin of replication,
b. a second positive selection marker,
c. a third Type IIS sequence,
d. TCR Joining gene segment,
e. a C part, corresponding to a small 5’ portion of a constant gene segment, and
f. a fourth Type IIS sequence.

7. A two-part device according to any of claims 5-6, wherein the 5’ genetic element of component **1A** further comprises one or more elements selected from
    a. gene cis/acting element,
    b. heterospecific recognition site for recombinase enzymes,
    c. a 5’ homologous recombination arm for a genomic site of interest,
    d. a mRNA splice acceptor site,
    e. an internal ribosomal entry site, and
    f. epigenetic insulator sequence,
    g. wherein the 5’ genetic element must contain at least b or c.

8. A two-part device according any of claims 6-7, wherein the negative selection marker in component **1A** is selected from one or more of the following
    a. a restriction enzyme recognition site not contained elsewhere in the first component or within the TCR joining gene segment,
    b. a gene encoding a conditional bactericidal agent, and
    c. a reporter element.

9. A two-part device according to any of claims 5-7, wherein the 3’ genetic element of component **1A** further comprises one or more elements selected from
        a. a terminator element,
        b. heterospecific recognition site for recombinase enzymes,
        c. a 3’ homologous recombination arm for a genomic site of interest,
        d. a mRNA splice donor site,
        e. an internal ribosomal entry site, and
        f. epigenetic insulator sequence,

wherein the 3’ genetic element must contain at least b or c.

10. A two-part device according to any of claims 5-9, wherein the first and second positive selection markers of the first part are different and are selected from an antibiotic resistance gene and/or auxotroph complementing gene.

11. A two-part device according to any of the preceding claims, wherein the first part further comprises a third component **1C** comprising an oligonucleotide duplex encoding CDR3 (odeCDR3).

12. A two-part device according to claim 11, wherein 1C comprises
    a. a first single strand overhang sequence complimentary to first Type IIS restriction enzyme recognition and cleavage site in **1A**,
    b. a double strand segment encoding a TCR CDR3 region and devoid of negative selection element in **1A**, wherein a negative selection element is devoid of any Type IIS restriction sequences of component **1A or 1B**, and
    c. a second single strand overhang sequence complimentary to the third Type IIS restriction enzyme recognition and cleavage site in **1B;**

    or 1C comprises

    d. a single double-stranded DNA molecule encoding a TCR CDR3 flanked by Type IIS restriction enzyme sites such that when cleaved generates the molecule defined in claims 12a to c.

13. A two-part device according to claim 1, wherein component 2**A** is a cell that lacks endogenous surface expression of at least one family of analyte antigen-presenting complexes (**aAPX**) and/or analyte antigenic molecule (**aAM**).

14. A two-part device according to **claim 13,** wherein the family of **aAPX** may be any of the following

    a. HLA class I

    b. HLA class II

    c. non-HLA antigen-presenting complex.

15. A two-part device according to **claim 1** wherein
    component 2**A**

a. Lacks endogenous expression of TCR chains alpha, beta, delta and gamma, and

b. Expresses CD3 proteins which are conditionally presented on the surface of the cell only when the cell expresses a complementary pair of TCR chains and

c. Contains a further component **designated 2B**, a genomic receiver site for integration of a single ORF encoding at least one analyte TCR chain of alpha, beta, delta or gamma, and/or two ORFs encoding pair of analyte TCR chains,

and component 2**C** comprises a genetic integration vector that is matched to component 2**B**, and wherein component 2**C** is designed to deliver

a. A single ORF encoding at least one analyte TCR chain of alpha, beta, delta and/or gamma and/or

b. Two ORFs encoding a pair of analyte TCR chains

and wherein a and/or b optionally encodes a selection marker of integration, such that the analyte TCR chains can be expressed as TCR surface protein in complex with the CD3 (**TCRsp**) on component 2**A.**

16. A two-part device according to **any of claims 1 and 15** wherein component 2**A**, further contains a component **designated 2F**, a synthetic genomic TCR-stimulation response element selected from

a. A single component synthetic construct containing at least one native promoter and/or at least one synthetic promoter and at least one reporter

b. A multi-component synthetic construct designed with at least one native promoter and/or at least one synthetic promoter and at least one reporter,

and wherein activation of a and/or b is dependent on at least one signal transduction pathway selected from a synthetic pathway, a native pathway or a combination thereof.

17. A two-part device according to claim 1, wherein the genomic receiver site 2B and 2D is a synthetic construct designed for recombinase mediated cassette exchange (RMCE).

18. A two-part device according to **claim 13-14,** wherein the **aAM** is selected from

a. a polypeptide or complex of polypeptides translated from the analyte antigenic molecule ORF(s)

b. a peptide derived from a polypeptide translated from the analyte antigenic molecule ORF(s)

c. a peptide derived from altering the component A proteome

d. a polypeptide derived from altering the component A proteome

e. a metabolite derived from altering the component A metabolome.

19. A two-part device according to any of **claims** 13, 14 and 18, wherein component 2**A** expresses CD4 and/or CD8.

20. A two-part device according to any of **claims 13-14 and 18-19,** wherein component 2**A** expresses additional TCR co-receptors.

21. A two-part device according to any of **claims 13-14 and 18-20,** wherein component 2**A** expresses CD28 and/or CD45.

22. A two-part device according to **any of claims 14-21,** wherein **component 2B and 2D** is included and comprises of at least one of the following genetic elements

a. Heterospecific recombinase sites

b. Homologous arms

c. Eukaryotic promoter

d. Eukaryotic conditional regulatory element

e. Eukaryotic terminator

f. Selection marker

g. Splice acceptor site

h. Splice donor site

i. Non-protein coding gene

j. Insulator

k. Mobile genetic element

l. Meganuclease recognition site

m. Internal ribosome entry site (IRES)

n. Viral self-cleaving peptide element

o. A Kozak consensus sequence.

23. A two-part device according to any of **claims 13-22,** wherein the **component 2C and 2E are** included and comprise of at least one of the following genetic elements

a. Heterospecific recombinase sites

b. Homologous arms

c. Eukaryotic promoter

d. Eukaryotic conditional regulatory element

e. Eukaryotic terminator

f. Selection marker

g. Selection marker of integration

h. Splice acceptor site

i. Splice donor site

j. Non-protein coding gene

k. Insulator

l. Mobile genetic element

m. Meganuclease recognition site

n. Internal ribosome entry site (IRES)

o. A viral self-cleaving peptide element

p. An antibiotic resistance cassette

q. A bacterial origin of replication

r. A yeast origin of replication

s. A cloning site

t. A kozak consensus sequence.

24. A two-part device according to **any of claims 14-23**, wherein the component **2B** and **2D are** included and are for RMCE integration of a single ORF and comprises:

    a. A Eukaryotic promoter

    b. A pair of heterospecific recombinase sites matched with those of 2C and 2E

    c. A Kozak consensus sequence

    d. A selection marker

    e. A Eukaryotic terminator.

25. A two-part device according to **any of claims** 14-24, wherein component **2C and 2E** are present and are each for RMCE integration of a single ORF and comprise the following genetic elements contributed by component **1A**:

    a. A pair of heterospecific recombinase sites matched with those of 2B and 2D

    b. A Kozak consensus sequence

    c. A TCR ORF reconstituted by operation of the Part 1 TORES.

26. A two-part device according to **any of** claims 14-25, wherein component **2C** and **2E** are included and are contributed by operation of the TORES to provide a single analyte TCR chain pair.

27. A two-part device according to **any of** claims 14-26, wherein component **2C** and/or **2E** are included and are contributed by operation of the TORES to provide a library of analyte TCR chain pairs encoded by **2C** and/or **2E**.

28. A two-part device according to claim 26 or 27 wherein the analyte TCR chain encoding sequences are derived from

a. Paired sequencing of TCR chain ORF sequence(s) from primary T-cells and reconstitution in the part 1 TORES

b. Unpaired sequencing of TCR chain ORF sequence(s) from primary T-cells and reconstitution in part 1 TORES

c. Synthetic TCR chain ORF sequence(s) generated by operation of the TORES.

29. A two-part device according to any of **claims 26-28** wherein one or more component **2C** and/or **2E** is combined with component **2A**, to integrate two complementary analyte TCR chains encoded in component **2C** and/or **2E**, into components **2B** and/or **2D**, to obtain a cell, **designated an eTPC-t,** wherein components **2B** and/or **2D** become components **2B'** and/or **2D'** such that it expresses an analyte **TCRsp** on the surface of component **2A**.

30. A two-part device according to any of **claims 26-28,** wherein one of component 2C or 2E is combined with component A, to integrate one analyte TCR chain encoded in component 2C or 2E, into components 2**B** or 2**D**, to obtain a cell, **designated eTPC-x,** wherein components B or D become components 2B' or 2D' such that the eTPC-x expresses a single TCR chain.

31. A two-part device according to any of **claim 30,** wherein one of component 2C or 2E is combined with an **eTPC-x**, to integrate one analyte TCR chain encoded in component 2C or 2E that is complementary to the TCR chain expressed in the **eTPC-x**, into components 2**B** or 2**D**, of the **eTPC-x**, to obtain an **eTPC-t,** wherein components 2B or 2D become components 2B' or 2D' such that it expresses an **TCRsp** on the surface of the eTPC-t.

32. A two-part device according to any of claims 1-31 for use in generating at least one analyte eTPC-t.