We claim:

1. A vaccine composition for prophylaxis against Hand Foot and Mouth Disease caused by Enterovirus -71 (EV71) and Coxsackievirus A16 (CVA16) comprising:
    a. a vaccine antigen, that is a synthetic construct selected from recombinant protein sequences as represented by SEQ ID No. 2 (named as MEV1) or SEQ ID No. 4 (named as MEV2);
    b. at least one adjuvant;
    c. at least one stabilizer; and
    d. a physiologically acceptable buffer selected from a phosphate or a citrate buffer;

wherein the said vaccine composition is stable for at least 2 years at 5 ±3^{o}C and up to 2 weeks at 37^{o}C.

2. The vaccine composition according to claim 1, wherein SEQ ID 2 is obtained from a codon optimized gene sequence as represented by SEQ ID No. 1.

3. The vaccine composition according to claim 1, wherein SEQ ID 4 is obtained from a codon optimized gene sequence as represented by SEQ ID No. 3.

4. The vaccine composition according to claim 1, wherein the at least one adjuvant is alum, a squalene-based adjuvant, a montanide, a RIBI adjuvant, a complete Freud's adjuvant, an incomplete Freud's adjuvant, MPL, muramyl dipeptide, an oil-in-water emulsion, a TLR ligand-based adjuvant, a CpG oligonucleotide, a non-CpG oligonucleotide, a saponin, a vitamin, or an immunomodulant.

5. The vaccine composition according to claim 1, wherein the at least one stabilizer is a sugar or a sugar alcohol.

6. The vaccine composition according to claim 1, wherein the said vaccine antigens SEQ ID No. 2 or SEQ ID No. 4 is prepared by a process comprising:
    a. expression of protein sequences SEQ ID No. 2 or SEQ ID No. 4 from codon optimized gene sequences, namely SEQ ID No. 1 and SEQ ID No. 3 respectively with 0.2-0.75 mM IPTG between 15 ^{o}C-37^{o}C, as inclusion bodies in a suitable host selected from *E. coli* BL21 (DE3) cells or Rosetta cells;

b. centrifugation at 10,000 rpm for 5 minutes to obtain MEV-1 or MEV-2 in a cell pellet;

c. wherein preparing the vaccine antigen SEQ ID No.2 comprises the steps of;

i) suspending the cell pellet of step (b) with a lysis buffer-I, the lysis buffer-I for MEV1 comprising 50mM Na_2HPO_4, 0.3M NaCl, 1% TritonX-114, 0.5mg/ml lysozyme, 1mM AEBSF, simultaneously performing freeze thaw cycles for at least 3 times followed by ultrasonication for 20 seconds thrice with at least 30 seconds intervals in between;

ii) undergoing cell lysis with a lysis buffer-II for MEV1, the lysis buffer-II for MEV1 comprising 3-6M Urea, 5-10mM imidazole, 50mM Na_2HPO_4, 0.3M NaCl, and 1mM AEBSF at 4ºC for 3-16 hours to recover at pH 7.4 an insoluble protein for MEV1 to obtain a denatured protein solution of MEV1;

iii) adding the denatured protein solution of MEV1 of step (ii) above to a Ni-NTA Immobilized Metal Affinity Chromatography column followed by washing with a Wash Buffer-I comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF, 3M urea at pH 7.4 with 0.1% Triton X-114 and 5-10 mM Imidazole;

iv) washing the column from step (iii) with a Wash Buffer–II comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF, 3-6M urea at pH 7.4 with 0.1% Triton X-114 and 20mM imidazole; followed by washing by a Wash Buffer – III, the Wash Buffer-III comprising 50 mM Na_2HPO_4, 0.3 M NaCl, 1 mM AEBSF additionally with 20mM imidazole with decreasing concentrations of urea;

v) eluting MEV1 from the column in step (iv), with an elution buffer the elution buffer comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF by using increasing concentrations of imidazole (250-500 mM) optionally with 3-6 M urea;

vi) undergoing dialysis of washed Ni-NTA column-based affinity purified protein MEV-1 against phosphate buffer saline using 10 kDa cutoff, membrane and refolding with a refolding buffer comprising 0.1-0.3M NaCl, 10% glycerol and 0.2-0.5 M Arginine or 0.2-0.5 M Arginine-HCl optionally followed by size exclusion chromatography to obtain MEV-1 with at least 92% to 96% purity;

d) wherein preparing the vaccine antigen SEQ ID No.4 comprises the steps of:

i) suspending the cell pellet of step (b) undergoing lysis with lysis buffer-I for MEV2, comprising 50 mM Tris, 0.3 M NaCl, 1% Triton X114, 0.5 mg/ml lysozyme, 1mM AEBSF at pH 8-8.5;

ii) or, alternatively, suspending the cell pellet of step (b) undergoing cell lysis with lysis buffer-II for MEV2, the lysis buffer-II for MEV2 comprising 50 mM Tris, 0.3 M NaCl, 1 mM AEBSF in presence of 6M urea and 10-20 mM DTT;

iii) undergoing refolding of MEV-2 after step (ii) above in decreasing concentration of urea and DTT concentration in presence of 02-1M Arginine or Arginine-HCL, redox pair reagents selected from Cysteine/Cystine, GSSG/GSH, oxidized DTT/reduced DTT or cystamine/cysteamine with concentration between 0.05mM to 10mM to obtain MEV-2 with at least 96% purity.

e) optionally storing MEV1 or MEV2 in a solution comprising polyols or sugars selected from 5-40% glycerol, 5-60% sucrose, 5-40% trehalose or 5-40% sorbitol at -20 degree C wherein the solution protects MEV1 or MEV2 from thermal stress.

7. A method of preparing the vaccine antigens SEQ ID No. 2 or SEQ ID No. 4 according to claim 1 comprising the steps of:

a. expressing protein sequences SEQ ID No. 2 or SEQ ID No. 4 from codon optimized gene sequences, namely SEQ ID No. 1 and SEQ ID No. 3 respectively with 0.2-0.75 mM IPTG between 15 ^{o}C-37^{o}C, as inclusion bodies in a suitable host selected from *E. coli* BL21 (DE3) cells or Rosetta cells;

b. centrifuging at 10,000 rpm for 5 minutes to obtain MEV1 or MEV2 in a cell pellet;

c. wherein preparing the vaccine antigen SEQ ID No.2 comprises the steps of;

i) suspending the cell pellet of step (b) with lysis buffer-I, the lysis buffer-I for MEV1 comprising 50mM Na_2HPO_4, 0.3M NaCl, 1% TritonX-114, 0.5mg/ml lysozyme, 1mM AEBSF, simultaneously performing freeze thaw cycles for at least 3 times followed by ultrasonication thrice for 20 seconds with at least 30 seconds intervals in between;

ii) undergoing cell lysis with a lysis buffer-II for MEV1, the lysis buffer-II for MEV1 comprising 3-6M Urea, 5-10mM imidazole, 50mM Na_2HPO_4, 0.3M

NaCl, and 1mM AEBSF at 4ºC for 3-16 hours to recover at pH of 7.4 an insoluble protein for MEV1 to obtain a denatured protein solution of MEV1;

iii) adding the denatured protein solution of MEV1 of step (ii) above to Ni-NTA Immobilized Metal Affinity Chromatography column followed by washing with a wash buffer-I comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF, 3M urea at pH7.4 with 0.1% Triton X-114 and 5-10 mM Imidazole;

iv) washing the column from step (iii) with a Wash Buffer–II comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF, 3-6M urea at pH7.4 with 0.1% Triton X-114 and 20mM imidazole; followed by washing with a Wash Buffer – III, the Wash Buffer-III comprising 50 mM Na_2HPO_4, 0.3 M NaCl, 1 mM AEBSF additionally with 20mM imidazole with decreasing concentrations of urea;

v) eluting the MEV1 from the column in step (iv) with an elution buffer, the elution buffer comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF by using increasing concentrations of imidazole (250-500 mM) optionally with 3-6 M urea;

vi) undergoing dialysis of MEV-1 against a phosphate buffer saline using 10 kDa cutoff, membrane and refolding with a refolding buffer comprising 0.1-0.3M NaCl, 10% glycerol and 0.2-0.5 M Arginine or 0.2-0.5 M Arginine-HCl optionally followed by size exclusion chromatography to obtain MEV-1 with at least 92% to 96% purity;

d) wherein preparing the vaccine antigen SEQ ID No.4 comprises the steps of;

i) suspending the cell pellet of step (b) undergoing lysis with a lysis buffer-I for MEV2 comprising 50 mM Tris, 0.3 M NaCl, 1% Triton X114, 0.5 mg/ml lysozyme, 1mM AEBSF at pH 8-8.5;

ii) or, alternatively undergoing cell lysis with lysis buffer-II for MEV2, the said lysis buffer-II for MEV2 comprising 50 mM Tris, 0.3 M NaCl, 1 mM AEBSF in presence of 6M urea and 10-20 mM DTT;

iii) undergoing refolding of MEV-2 after step (ii) above in decreasing concentration of urea (from 6 M to 0.1 M) and DTT concentration (from 20 mM to 0.1 mM) in presence of 0.2-1M Arginine or Arginine-HCL, redox pair reagents selected from Cysteine/Cystine, GSSG/GSH, oxidized DTT/reduced DTT or cystamine/cysteamine with concentration between 0.05mM to 10mM to MEV-2 with at least 96% purity.

e) optionally storing MEV1 or MEV2 in a solution comprising a polyol or a sugar, selected from 5-40% glycerol, 5-60% sucrose, 5-40% trehalose or 5-40% sorbitol at -20 degree C to protect MEV1 or MEV2 from thermal stress.

8. A vaccine composition comprising a multi-epitope synthetic protein as disclosed in SEQ ID No. 2 with at least 92% - 96% purity as determined by SDS-PAGE and Western blotting.

9. A vaccine composition comprising a multi-epitope synthetic protein as disclosed in SEQ ID no. 4 with at least 96 % purity as determined by SDS-PAGE and Western blotting.

10. The vaccine composition according to claim 4, wherein the alum is aluminium hydroxide.

11. The vaccine composition according to claim 4, wherein the saponin is QS-1, ISCOM, or ISCOMATRIX.

12. The vaccine composition according to claim 4, wherein the Immunomodulant is a cytokine.

13. The vaccine composition according to claim 4, wherein the squalene-based adjuvant is MF59.

14. The vaccine composition according to claim 5, wherein the sugar is 5-60% sucrose or 5-40% trehalose.

15. The vaccine composition according to claim 5, wherein the sugar alcohol is 5-40% glycerol or 5-40% sorbitol.