We claim:

1. A vaccine composition for prophylaxis against Hand Foot and Mouth Disease caused by EV71 and CA16 comprising:
   a. vaccine antigen, the said vaccine antigen is a synthetic construct selected from the recombinant protein sequences as represented by SEQ ID No. 2 (named as MEV1) and SEQ ID No. 4 (named as MEV2);
   b. adjuvants;
   c. stabilizers; and
   d. a physiologically acceptable buffer selected from phosphate and citrate;

wherein the said vaccine formulation is stable for at least 2 years at 5 ±3°C and up to 2 weeks at 37°C.

2. The vaccine composition as claimed in claim 1, wherein the vaccine antigen having protein SEQ ID 2 is obtained from a codon optimized gene sequence as represented by SEQ ID No. 1.

3. The vaccine composition as claimed in claim 1, wherein the vaccine antigen having protein SEQ ID 4 is obtained from a codon optimized gene sequence as represented by SEQ ID No. 3.

4. The vaccine composition as claimed in claim 1, wherein the adjuvant is selected from a group of alum (aluminium phosphate, aluminium hydroxide), squalene based adjuvants such as MF59, montanide, RIBI adjuvant, complete Freud's adjuvant and incomplete Freud's, MPL, muramyl dipeptide, oil-in-water emulsion, TLR ligand based adjuvants, CpG oligonucleotides, Non-CpG oligonucleotides, saponins such as QS-1, ISCOM, ISCOMATRIX, vitamins, and immunomodulants such as cyotkines.

5. The vaccine composition as claimed in claim 1, wherein the stabilizers are selected from sugar 5-60% sucrose or 5-40% Trehalose or sugar alcohols like 5-40% Glycerol or 5-40% Sorbitol .

6. The vaccine composition as claimed in claim 1, wherein the said vaccine antigens SEQ ID No. 2 and SEQ ID No. 4 is prepared by a process comprising:

a. expression of the protein sequences SEQ ID No. 2 and 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 °C-37°C, in the form of 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 the protein of interest in the cell pellet;

c. suspending the pellet of step (b) with lysis buffer-I, the said 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 with at least 30 seconds intervals in between;

d. alternatively undergoing lysis with lysis buffer-I for MEV2, the said lysis buffer-I for MEV1 comprising Lysis Buffer-I for MEV2 comprised 50 mM Tris, 0.3 M NaCl, 1% Triton X114, 0.5 mg/ml lysozyme, 1mM AEBSF at pH 8-8.5;

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

f. 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;

g. adding the denatured protein solution of step (e) above to Ni-NTA Immobilized Metal Affinity Chromatography column followed by washing with 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;

h. again performing washing after step (g) above for MEV1 with 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 by Wash Buffer – III, the said 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;

i. eluting the target protein MEV1 with elution buffer, the said elution buffer comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF by increasing concentrations of imidazole (250-500 mM) optionally with 3-6 M urea;

j. undergoing dialysis of MEV-1 after step (i) above against phosphate buffer saline using 10 kDa cutoff, membrane and refolding with refolding buffers 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 the final protein MEV-1 with at least 92% to 96% purity;

k. undergoing refolding of MEV-2 after step (f) above in decreasing urea and DTT concentration 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 obtain the final protein MEV-2 with at least 96% purity.

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

a. expression of the protein sequences SEQ ID No. 2 and 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 °C-37°C, in the form of 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 the protein of interest in the cell pellet;

c. suspending the pellet of step (b) with lysis buffer-I, the said 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 with at least 30 seconds intervals in between;

d. alternatively undergoing lysis with lysis buffer-I for MEV2, the said lysis buffer-I for MEV1 comprising Lysis Buffer-I for MEV2 comprised 50 mM Tris, 0.3 M NaCl, 1% Triton X114, 0.5 mg/ml lysozyme, 1mM AEBSF at pH 8-8.5;

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

f. 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;

g. adding the denatured protein solution of step (e) above to Ni-NTA Immobilized Metal Affinity Chromatography column followed by washing with 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;

h. again performing washing after step (g) above for MEV1 with 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 by Wash Buffer – III, the said 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;

i. eluting the target protein MEV1 with elution buffer, the said elution buffer comprising 50mM Na_2HPO_4, 0.3M NaCl, 1mM AEBSF by increasing concentrations of imidazole (250-500 mM) optionally with 3-6 M urea;

j. undergoing dialysis of MEV-1 after step (i) above against phosphate buffer saline using 10 kDa cutoff, membrane and refolding with refolding buffers 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 the final protein MEV-1 with at least 92% to 96% purity;

k. undergoing refolding of MEV-2 after step (f) above in decreasing urea (from 6 M to 0.1 M) and DTT (from 20 mM to 0.1 mM) concentration 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 obtain the final protein MEV-2 with at least 96% purity.

8. A multi-epitope synthetic protein sequence as disclosed in SEQ ID No. 2 with at least 92% - 96% purity.

9. A multi-epitope synthetic protein sequence as disclosed in SEQ ID no. 4 with at least 96 % purity.