applied sciences

Supplementary Data

Optimization of imidazole concentration for purification of H5.c1 by IMAC

Proteins in the crude extract (CE), Flow through (FT), washing (W) and elution (E) were loaded into the wells for SDS–PAGE 10% at reducing conditions and detected by Western blot using anti-cmyc antibody and ECL analysis. A series of difference imidazole concentration (30, 25, 20, or 5 mM imidazole which are W30, W25, W20, or W5, respectively) have tested at the washing step. E30, E25, E20 or E5 are elution steps which the washing steps using imidazole 30, 25, 20, or 5 mM, respectively. W5 are the most optimized concentration have chosen for purifying H5.c1 (B, C). 5 µg of purified H5TG protein using as a positive control (+) (Figure S1).

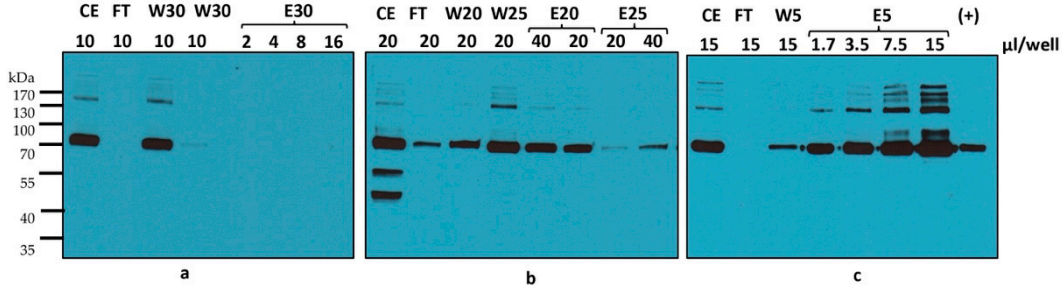

Figure S1. Optimization of imidazole concentration for purification of H5.c1 by immobilized metal ion affinity chromatography (IMAC).

Purification of H5.c1 and H5TG by Size Exclusion Chromatography (SEC)

2.5 mg/ml of purified H5.c1 and H5TG collected after purification by IMAC were applied on a SuperoseTM 6 increase 10/300GL column which was pre-equilibrated with phosphate-buffered saline (PBS). The fractions from B1 to B6 were collected for concentrating and detected by SDS–PAGE and Coomassie stain. 2 and 2,5 µg of the purified H5.c1 and H5TG proteins obtained from SEC, respectively, were loaded on 10% SDS–PAGE and detected by Coomassie stain. M is protein marker (Figure S2).

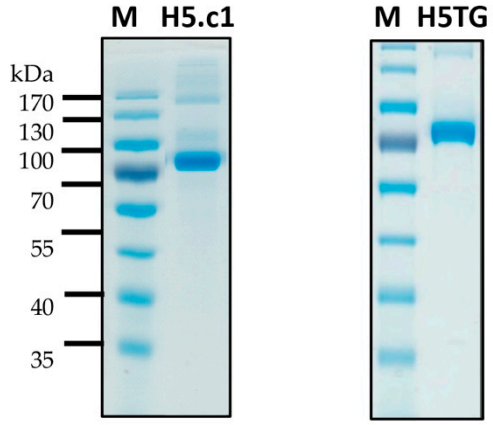

Figure S2. Purification of H5.c1 and H5TG by size exclusion chromatography (SEC).